NEPHROPROTECTIVE ACTIVITY OF *TAMARINDUS INDICA* LINN FRUIT EXTRACT ON CISPLATIN INDUCED NEPHROTOXICITY IN RATS

A Dissertation Submitted to

THE TAMIL NADU Dr. M.G.R. MEDICAL UNIVERSITY

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MASTER OF PHARMACY

IN

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Submitted by

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EVALUATION CERTIFICATE

This is to certify that the dissertation work entitled **"Nephroprotective activity of *Tamarindus indica* Linn fruit extract on Cisplatin induced nephrotoxicity in Rats"** submitted by **Register No: 261425505** to The Tamil Nadu Dr. M.G.R Medical University, Chennai, in partial fulfilment for the degree of **Master of Pharmacy** in **Pharmacology** is the bonafide work carried out under the guidance and direct supervision of **Mrs. G. Sumithira, M.Pharm., Assistant Professor** at the Department of Pharmacology, **The Erode College of Pharmacy and Research Institute**, **Erode-638112** and was evaluated by us during the academic year 2015-2016.

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DECLARATION

I do hereby declare that the dissertation work entitled **"Nephroprotective activity of *Tamarindus indica* Linn fruit extract on cisplatin induced nephrotoxicity in Rats"** submitted to The Tamil Nadu Dr. M.G.R Medical University, Chennai, in the partial fulfilment for the Degree of **Master of Pharmacy in Pharmacology**, was carried out by myself under the guidance and direct supervision of **Mrs. G. Sumithira, M.Pharm., Assistant Professor,** at the Department of Pharmacology, The Erode College of Pharmacy and Research Institute, Erode-638112, during the academic year 2015-2016.

This work is original and has not been submitted in part or full for the award of any other Degree or Diploma of this or any other University.

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LIST OF ABBREVATIONS

BC	:	Before Christ
WHO	:	World health organisation
PCT	:	proximal convoluted tubule
DCT	:	Distal convoluted tubule
ATP	:	Adinine triphosphate
ADH	:	Anti diuretic hormone
SLE	:	Systemic lupus erythematosus
MMF	:	Mychophenolate mofetil
IgA	:	Immunoglobulin A
ACE	:	Angiotensin converting enzyme
ARBs	:	Angiotensin receptor blockers
PSGN	:	Post-streptococcal glomerulonephritis
AIDS	:	Acquired immune deficiency syndrome
HIV	:	Human immune deficiency virus
mmHg	:	Millimeter mercury
FSGS	:	Focal segmental glomerulosclerosis
MCD	:	Minimal change disease
ARF	:	Acute renal failure
CKD	:	Chronic kidney disease
ESRD	:	End-stage renal disease
AKI	:	Acute kidney injury
BUN	:	Blood urea nitrogen
ADQI	:	Acute dialysis quality initiative
AKIN	:	Acute kidney injury network
KDIGO	:	Kidney disease international global outcome
SCr	:	Serum creatinine

PMP	:	Per million population per year
ICU	:	Intensive care unit
dRTA	:	Distal renal tubular acidosis
CIN	:	Contrast induced nephropathy
DNA	:	Deoxy ribonucleic acid
RNA	:	Ribo nuclic acid
ROS	:	Reactive oxygen species
DAMPs	:	Damage-associated molecular pattern molecules
TLR4	:	Toll-like receptor 4
TNF-α	:	Tumour necrosis factor alpha
CMV	:	Cytomegalovirus
CT Scan	:	Computed tomography scan
MRI	:	Magnetic resonance imaging
rhEPO	:	Erythropoitin
%	:	percentage
ml /kg	:	Milli litre per kilogram
im	:	Intra muscular
v/v	:	volume by volume
hs	:	Hours
mg/kg	:	Milli gram per kilo gram
MPTP	:	Mitochondrial permeability transition pore
w/v	:	Weight by volume
GFR	:	Glomourular filtration rate
CM	:	Contrast media
CYP	:	Cytochrome phosphate
GSH	:	Reduced glutathione
Ip	:	Intra peritoneal

NSAID	:	Non-steroidal anti inflammatory drug
IFO	:	Ifosfamide
UN	:	Uranium nitrate
Nacl	:	Sodium chloride
$HGCl_2$	:	Mercuric chloride
$K_2Cr_2O_7$	:	Potassium dichromate
FA	:	Folic acid
Bcl-xL	:	B-cell lymphoma-extra large
NTA	:	Nitrilotriacetic acid
Fe-NTA	:	Ferric- Nitrilotriacetic acid
DCVC	:	S-(1,2-dichlorovinyl)-L-cysteine
TCE	:	Trichloroethylene
ATSDR	:	Agency of Toxic Substances and Disease Registry
LPS	:	Lipopolysaccaride
CLP	:	Cecal ligation and puncture
MDA	:	Malondialdehyde
SOD	:	Superoxide dismutase
GPx	:	Glutathione peroxidise
L.	:	Linn
LPO	:	Lipid peroxidation
CAT	:	Catalase
SGOT	:	Serum glutamic oxaloacetic transaminase
SGPT	:	Serum glutamic pyruvic transaminase
Fig.	:	Figure
OECD	:	Organisation for economic and cultural development
STZ	:	Streptozotocin

EETI	:	Ethanolic extract of *Tamarindus indica* Linn
ANOVA	:	Analysis of variance
DMSO	:	Dimethyl sulphoxide

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1. INTRODUCTION

Plants are essential components of the universe. After various observations and experimentations many medicinal plants were identified as source of important medicine. Medicinal plants have been used since prehistoric period for the cure of various diseases.[1]

About 8,000 herbal remedies have been described in Ayurveda. The Rig-Veda (5000 BC) has recorded 67 medicinal plants, Yajurveda 81 species, Atharvaveda (4500-2500 BC) 290 species, Charak Samhita (700 BC) and Sushrut Samhita (200 BC) had described properties and uses of 1100 and 1270 species respectively. In compounding of drugs and these are still used in the classical formulations and in the Ayurvedic system of medicine. From time immemorial man depended on plants derived medicines, it is evident that the fascination for plants is as old as mankind itself. The plant kingdom represents a rich storehouse of organic compounds, many of which have been used for medical purpose and could serve as lead for the development of novel agents having good efficacy in various pathological disorders.[2]

The World Health Organization (WHO) estimates that about 80% of population living in the developing countries relies on traditional medicines for their primary health care needs. In almost all the traditional system of medicines, the medicinal plants play a major role and constitute the backbone. A large body of evidence has collected to show potential of medicinal plants used in various traditional systems. In the last few years more than 13 000 plants have been studied for the various diseases and ailments all over the world.[3]

Herbal medicine is the study and use of medicinal properties of plants. Plants have the ability to synthesize a wide variety of chemical compounds that are used to perform important biological functions. Most of the phytochemical beneficial effects consumed by human beings, they are used effectively treat to human disease. At least 12,000 such compounds have been isolated so far; a number estimated to be less than 10% of the total. Chemical compounds in plants mediated their effects on the human body through processes identical to those already well understood for the chemical compounds in conventional drugs; thus herbal medicines do not differ

greatly from conventional drugs in terms of how they work. This enables herbal medicines to be as effective as curative medicines.[4]

In general, traditional practitioners used crude drug either in fresh juice or decoction for healing various ailments. The medicinal plants belonging to established system of medicine i.e. Ayurveda, Unani and Siddha were used by the traditional folk healers. They used crude drugs in single or combination of drugs for the treatment of different diseases.

Considerable research on Pharmacognosy, Chemistry, Pharmacology and Clinical therapeutics has been carried out on native medicinal plants. Traditional knowledge driven drug development can follow a reverse pharmacology path and reduce time and cost of development.

In Indian system of medicine several herbal remedies has been tried for the treatment of kidney failure since the time of charka and sushruta. New approaches to improve and accelerate the joint drug discovery and development process are expected to take place mainly from innovation in drug target elucidation and lead structure discovery.[5]

Extracts and metabolites of the plant particularly those from leaves and fruits possess useful pharmacological activities. The fruits are utilized as vegetable and regarded as essential ingredient in the South Indian diet.[6, 7]

Ethnobotanical studies are often significant in revealing locally important plant species especially for the discovery of crude drug.[8]

Allopathy is a system of medicines with full of side effects, in lay man's words but any how this has become a synonym for evidence based system of medicine or modern medicine. Allopathy is totally a system of physical health and moreover this science has become a bio-engineering and bio-mechanical system of medicines, where they believe to replace or change of the organs or systems in name of treatment, not much worried about the cure. The allopathic medicines keep on suppressing the signs and symptoms alone, not concerned with the Root Cause of a disease. As modern sciences believe in suppressing the signs and symptoms, these never appreciate to remove the disease causing factors from the body. Due to these disadvantages of allopathic medicines nowadays the herbal drugs are mostly preferred than the allopathic medicines.[9]

Traditional knowledge will serve as a powerful search engine and most importantly, will greatly facilitate intentional, focused and safe natural product research to rediscover the drug discovery process. Therefore, search of Nephroprotective herbs from medicinal plants has become important and need of the day.[10]

Considering this situation, I selected *Tamarindus indica linn* for the evaluation of its Nephroprotective activity.

2. REVIEW OF LITERATURE

2.1 KIDNEY-INTRODUCTION

The kidney is an excretory organ. It is located on the posterior abdominal wall, one on each side of the lumbar part of the vertebral column. They reside against the back muscles in the upper abdominal cavity. They sit opposite each other on either side of the spine. The right kidney sits a little bit lower than the left to accommodate the liver.[11]

The formation of urine is achieved through the processes of filtration, re-absorption, and secretion by the glomeruli and tubules within the kidney. The bladder stores urine that it received from the kidney by the way of ureters. Urine is then excreted from the body through urethra.

- The main function of the kidney is excretion of waste products like urea, uric acid, creatinine, etc.
- It also regulates the blood contents of Nacl and other electrolytes, as well as the volume of extracellular fluid to maintain homeostasis and regulate blood pressure.
- It plays a crucial role in maintaining acid-base balance.
- It is responsible for re-absorption of water, glucose and amino acids.
- It helps with synthesis of local hormones like angiotensin, prostacyclin and erythropoietin.
- It is also involved in the hydroxylation of vitamin D.
- It also eliminates the poisons from the blood.
- It is the major excretory part for drug metabolites.

NEPHRON

The Nephron is the basic structural and functional unit of the kidney.[12] Its functions are vital to life and are regulated by the endocrine system by hormones such as antidiuretic hormone, aldosterone, thyroid and parathyroid hormone.[13] In humans, a normal kidney contains 800,000 to 1.5 million of Nephrons.[14]

2.1.1 ANATOMY OF NEPHRON

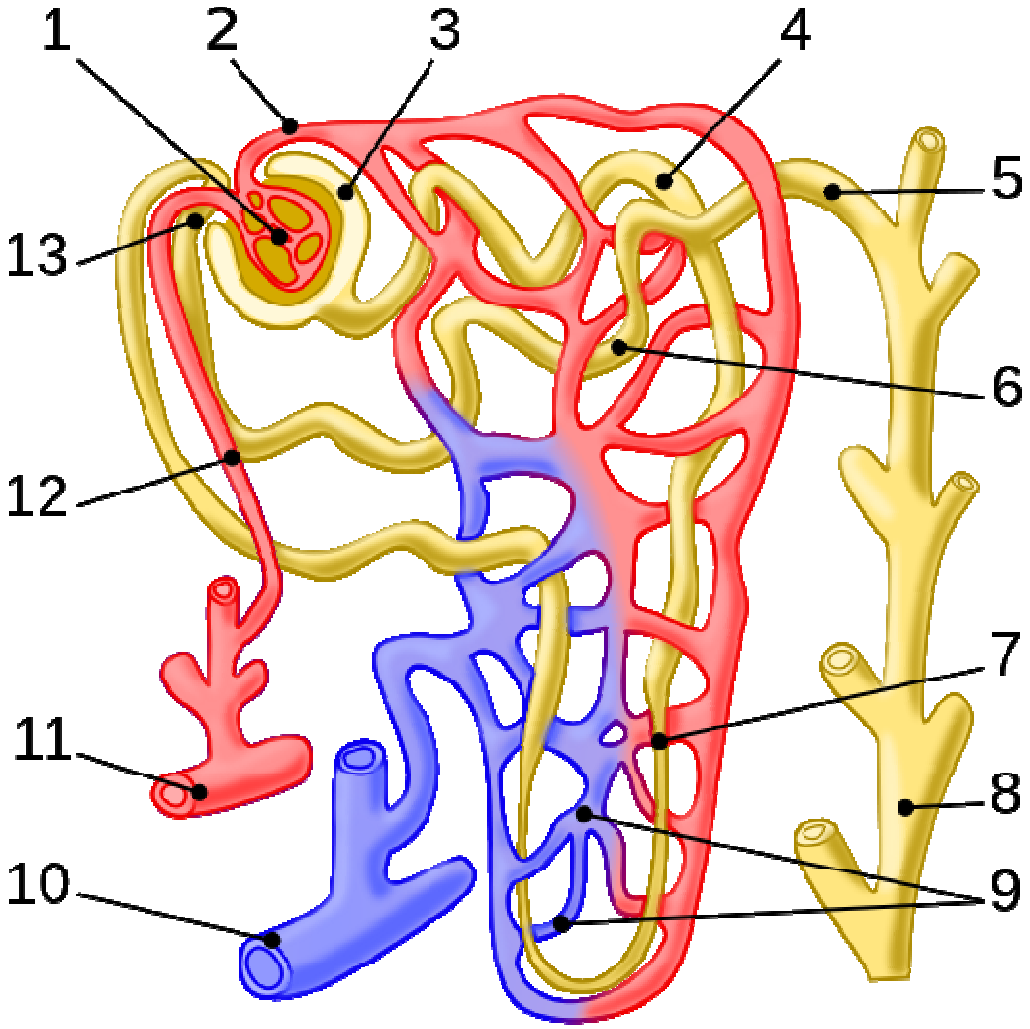


Fig.1: Anatomy of Nephron

The labelled parts are,

1. Glomerulus, 2. Efferent arteriole, 3. Bowman's capsule, 4. Proximal convoluted tubule, 5. Cortical collecting duct, 6. Distal convoluted tubule, 7. Loop of Henle, 8. Papillary duct, 9. Peritubular capillaries, 10. Arcuatevein, 11. Arcuateartery, 12. Afferent arteriole, 13. Juxtaglomerular apparatus.

2.1.2 HISTOLOGY[15]

Nephron histology studies the structure of the Nephron as viewed under a microscope. Various distinct cell types occur in the Nephron, which include:

Each Nephron is composed of an initial filtering component (the "renal corpuscle") and a tubule specialized for re-absorption and secretion (the "renal tubule"). The renal corpuscle filters out solutes from the blood, delivering water and small solutes to the renal tubule for modification.

Renal corpuscle

It is composed of a glomerulus and Bowman's capsule, the renal corpuscle (or Malpighian corpuscle) is the beginning of the Nephron. It is the Nephron's initial filtering component.

Glomerulus

The glomerulus is a capillary tuft that receives its blood supply from an afferent arteriole of the renal circulation. The glomerular blood pressure provides the driving force for water and solutes to be filtered out of the blood and into the space made by Bowman's capsule.

The remaining blood (only approximately 1/5 of all plasma passing through the kidney is filtered through the glomerular wall into the Bowman's capsule) passes into the efferent arteriole. The diameter of efferent arterioles is smaller than that of afferent arterioles, increasing the hydrostatic pressure in the glomerulus.

Bowman's capsule

The Bowman's capsule, also called the glomerular capsule, surrounds the glomerulus. It is composed of a visceral inner layer formed by specialized cells called podocytes, and a parietal outer layer composed of simple squamous epithelium. Fluids from blood in the glomerulus are filtered through the visceral layer of podocytes, resulting in the glomerular filtrate.

The glomerular filtrate then moves to the renal tubule, where it is further processed to form urine. The different stages of this fluid are collectively known as the tubular fluid.

Renal tubule

The renal tubule is the portion of the Nephron containing the tubular fluid filtered through the glomerulus.[16] After passing through the renal tubule, the filtrate continues to the collecting duct system.[17]

The components of the renal tubule are:

- **Proximal convoluted tubule** (lies in cortex and lined by simple cuboidal epithelium with brush borders which help to increase the area of absorption greatly.)
- **Loop of Henle** (hair-pin like i.e. U-shaped and lies in medulla)
- Descending limb of loop of Henle

  The descending limb of loop of Henle is divided into 2 segments:

  - Thick descending limb of loop of Henle (Begins from PCT-proximal convoluted tubule.)
  - Thin descending limb of loop of Henle
- Ascending limb of loop of Henle

  The ascending limb of loop of Henle is divided into 2 segments: Lower end of ascending limb is very thin and is lined by simple squamous epithelium. The distal portion of ascending limb is thick and is lined by simple cuboidal epithelium.

  - Thin ascending limb of loop of Henle
  - Thick ascending limb of loop of Henle (enters cortex and becomes DCT-distal convoluted tubule.)
- **Distal convoluted tubule**

Blood from the efferent arteriole containing everything that was not filtered out in the glomerulus, moves into the peritubular capillaries tiny blood vessels that surround the loop of Henle and the proximal and distal tubules, where the tubular fluid flows. Substances then reabsorb from the latter back to the blood stream.

The peritubular capillaries then recombine to form an efferent venule, which combines with efferent venules from other Nephrons into the renal vein, and rejoins the main bloodstream.

2.1.3 Classes of Nephron

There are two general classes of Nephrons. They are,

a) **Cortical Nephrons**

b) **Juxtamedullary Nephrons**

Both of which are classified according to the length of their associated Loop of Henle and location of their renal corpuscle. All Nephrons have their renal corpuscles in the cortex. Cortical Nephrons have their Loop of Henle in the renal medulla near its junction with the renal cortex, while the Loop of Henle of juxtamedullary Nephrons is located deep in the renal medulla; they are called juxtamedullary because their renal corpuscle is located near the medulla (but still in the cortex). The nomenclature for cortical Nephrons varies, with some sources distinguishing between superficial cortical Nephrons and midcortical Nephrons, depending on where their corpuscle is located within the cortex.[18]

The majority of Nephrons are cortical. Cortical Nephrons have a shorter loop of Henle compared to juxtamedullary Nephrons. The longer loop of Henle in juxtamedullary Nephrons create a hyperosmolar gradient that allows for the creation of concentrated urine.[19]

The peritubular capillaries surround the proximal and distal tubules in both classes of Nephrons. However, they surround the loop of Henle, forming the vasa recta, only in juxtamedullary Nephrons and not in cortical Nephrons.

2.1.4 Functions of Nephron[15]

The Nephrons carried out nearly all of the kidney's function. Most of these functions are reabsorption and secretion of various solutes such as ions, carbohydrates and amino acids.

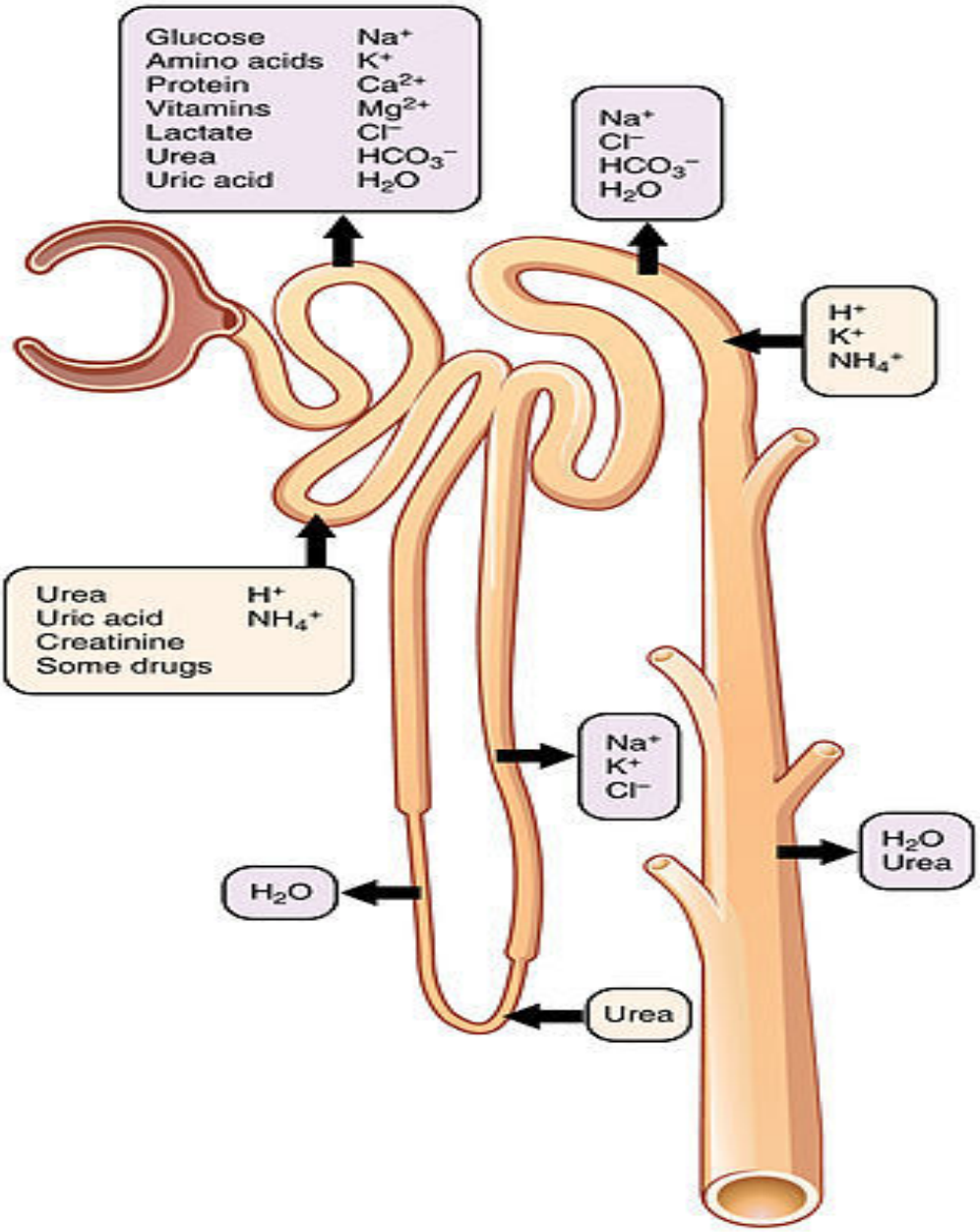


Fig.2: Functions of Nephron

The major functions of Nephrons are,

- Regulation of the concentration of water and soluble substances like sodium salts by filtering the blood
- Re-absorption of what is needed and excreting the rest as urine.
- Elimination of wastes from the body
- Regulates blood volume
- Regulates blood pressure
- Controls levels of electrolytes and metabolites
- Regulates blood pH.

Each segment of the Nephron has highly specialized functions.

2.1.4.1 Functions of Proximal convoluted tubule

Fluid in the filtrate entering the proximal convoluted tubule is reabsorbed into the peritubular capillaries, including approximately two-thirds of the filtered salt and water and all filtered organic solutes (primarily glucose and amino acids).

2.1.4.2 Functions of loop of Henle

The loop of Henle is a U-shaped tube that extends from the proximal tubule. It consists of a descending limb and an ascending limb. It begins in the cortex, receiving filtrate from the proximal convoluted tubule, extends into the medulla as the descending limb, and then returns to the cortex as the ascending limb to empty into the distal convoluted tubule.

The primary role of the loop of Henle is to concentrate the salt in the interstitium, the tissue surrounding the loop.

Considerable differences aid in distinguishing the descending and ascending limbs of the loop of Henle. The descending limb is permeable to water and noticeably less impermeable to salt, and thus only indirectly contributes to the concentration of the interstitium. As the filtrate descends deeper into the hypertonic interstitium of the renal medulla, water flows freely out of the descending limb by osmosis until the tonicity of the filtrate and interstitium equilibrate.

The hypertonicity of the medulla (and therefore concentration of urine) is determined in part by the size of the loop of Henle.

Unlike the descending limb, the thin ascending limb is impermeable to water, a critical feature of the counter current exchange mechanism employed by the loop. The ascending limb actively pumps sodium out of the filtrate, generating the hypertonic interstitium that drives counter current exchange. In passing through the ascending limb, the filtrate grows hypotonic since it has lost much of its sodium content. This hypotonic filtrate is passed to the distal convoluted tubule in the renal cortex.

2.1.4.3 Functions of distal convoluted tubule

The distal convoluted tubule has a different structure and function to that of proximal convoluted tubule. Cells lining the tubule have numerous mitochondria to produce enough energy (ATP) for active transport to take place. Much of the ion transport taking place in the distal convoluted tubule is regulated by the endocrine system. In the presence of parathyroid hormone, the distal convoluted tubule reabsorbs more calcium and secretes more phosphate. When aldosterone is present, more sodium is reabsorbed and more potassium secreted. Atrial natriuretic peptide causes the distal convoluted tubule to secrete more sodium. In addition, the tubule also secretes hydronium and ammonium cations to regulate urine pH.

2.1.4.4 Functions of Collecting duct system

Each distal convoluted tubule delivers its filtrate to a system of collecting ducts, the first segment of which is the connecting tubule. The collecting duct system begins in the renal cortex and extends deep into the medulla. As the urine travels down the collecting duct system, it passes by the medullary interstitium which has a high sodium concentration as a result of the loop of Henle's counter current multiplier system.

Though the collecting duct is normally impermeable to water, it becomes permeable in the presence of antidiuretic hormone (ADH). ADH affects the function of aquaporins, resulting in the re-absorption of water molecules as it passes through the collecting duct. Aquaporins are membrane proteins that selectively conduct water molecules while preventing the passage of ions and other solutes. As much as 3/4 of the water from urine can be reabsorbed as it leaves the collecting duct by osmosis. Thus the levels of ADH determine whether urine will be concentrated or diluted. An

increase in ADH is an indication of dehydration, while water sufficiency results in a decrease in ADH allowing for diluted urine.

Lower portions of the collecting organ are also permeable to urea, allowing some of it to enter the medulla of the kidney, thus maintaining its high concentration (which is very important for the Nephron).

Urine leaves the medullary collecting ducts through the renal papillae, emptying into the renal calyces, the renal pelvis, and finally into the urinary bladder via the ureter. The collecting duct originates from the ureteric bud.

2.1.4.5 Functions of juxtaglomerular apparatus

The juxtaglomerular apparatus is a specialized region of the Nephron responsible for production and secretion of the enzyme renin, involved in the renin-angiotensin system. This occurs near the site of contact between the thick ascending limb and the afferent arteriole. It contains three components: Macula densa, Juxtaglomerular cells and Extraglomerular mesangial cells.

2.1.5 Pathology of Nephron (kidney)[20,21,22]

- **Nephrotic Syndrome**

The Nephrotic syndrome is a condition marked by very high levels of protein in the urine; low levels of protein in the blood; swelling, especially around the eyes, feet, and hands; and high cholesterol.

The Nephrotic syndrome is a set of symptoms, not a disease in itself. It can occur with many diseases, so prevention relies on controlling the diseases that cause it. Treatment of the Nephrotic syndrome focuses on identifying and treating the underlying cause, if possible, and reducing high cholesterol, blood pressure, and protein in the urine through diet, medication, or both.

The Nephrotic syndrome may go away once the underlying cause, if known, is treated. However, often a kidney disease is the underlying cause and cannot be cured. In these cases, the kidneys may gradually lose their ability to filter wastes and excess water from the blood. If kidney failure occurs, the patient will need to be on dialysis or have a kidney transplant.

- **Pyelonephritis** (Infection of kidney pelvis)

Bacteria may infect the kidney, usually causing back pain and fever. A spread of bacteria from an untreated bladder infection is the most common cause of pyelonephritis.

- **Polycystic kidney disease**

A genetic condition resulting in large cyst in the kidney that impair their functions.

- **Hypertensive nephropathy**

It is the damage of kidney caused by high blood pressure. Chronic renal failure may eventually result.

- **Kidney cancer**

Renal cell carcinoma is the most common cancer affecting the kidney. Smoking is the most common cause of kidney cancer.

- **Interstitial nephritis**

Inflammations on the connective tissues of the kidney, which often causing acute renal failure, allergic reactions and drug side effects are the usual causes.

- **Kidney stones** (Urolithiasis)

Minerals in the urine form crystals (stones), which may grow large enough to block urine flow. It is considered as one of the most painful conditions. Most kidney stones pass on their own but some are too large and need to be treated.

- **Glomerulonephritis**

It describes the inflammation of the membrane tissue in the kidney that serves as a filter, separating wastes and extra fluid from the blood.

- **Glomerulosclerosis**

It describes the scarring or hardening of the tiny blood vessels within the kidney.

- **Lupus nephritis**

It is the name given to the kidney disease caused by Systemic lupus erythematosus (SLE), and it occurs when auto-antibodies form or are deposited in the glomeruli, causing inflammation. Ultimately, the inflammation may create scars that keep the kidneys from functioning properly. Conventional treatment for lupus nephritis includes a combination of two drugs, cyclophosphamide, a cyotoxic agent that suppresses immune system, prednisolone, it is a corticosteroid used to reduce inflammation. A newer immunosuppressant, mychophenolate mofetil (MMF), has been used instead of cyclophosphamide. Preliminary studies indicate that MMF may be as effective as cyclophosphamide and has milder side effects.

- **Goodpasture's Syndrome**

It involves an autoantibody that specifically targets the kidneys and the lungs. Often, the first indication that patients have the autoantibody is when they cough up blood. But lung damage in Goodpasture's Syndrome is usually superficial compared with progressive and permanent damage to the kidneys. Goodpasture's Syndrome is a rare condition that affects mostly young men but also occurs in women, children, and older adults. Treatments include immunosuppressive drugs and a blood-cleaning therapy called plasmapheresis that removes the auto antibodies.

- **IgA nephropathy**

It is a form of glomerular disease that results when immunoglobulin A (IgA) forms deposits in the glomeruli, where it creates inflammation. IgA nephropathy was not recognized as a cause of glomerular disease until the late 1960s, when sophisticated biopsy techniques were developed that could identify IgA deposits in kidney tissue.

The most common symptom of IgA nephropathy is blood in the urine, but it is often a silent disease that may go undetected for many years. The silent nature of the disease makes it difficult to determine how many people are in the early stages of IgA nephropathy, when specific medical tests are the only way to detect it. It appears to affect men more than women. Although IgA nephropathy is found in all age groups, young people rarely display signs of kidney failure because the disease usually takes several years to progress to the stage where it causes detectable complications.

No treatment is recommended for early or mild cases of IgA nephropathy when the patient has normal blood pressure and less than 1 gram of protein in a 24-hour urine output.

When proteinuria exceeds 1 gram/day, treatment is aimed at protecting kidney function by reducing proteinuria and controlling blood pressure. Blood pressure medicines angiotensin converting enzyme inhibitors (ACE inhibitors) or angiotensin receptor blockers (ARBs) that block a hormone called angiotensin are most effective at achieving those two goals simultaneously.

- **Hereditary Nephritis-Alport Syndrome**

The primary indicator of Alport syndrome is a family history of chronic glomerular disease, although it may also involve hearing or vision impairment. This syndrome affects both men and women, but men are more likely to experience chronic kidney disease and sensory loss. Men with Alport syndrome usually first show evidence of renal insufficiency while in their twenties and reach total kidney failure by age 40. Women rarely have significant renal impairment, and hearing loss may be so slight that it can be detected only through testing with special equipment. Usually men can pass the disease only to their daughters. Women can transmit the disease to either their sons or their daughters. Treatment focuses on controlling blood pressure to maintain kidney function.

❖ **Infection-related Glomerular Disease**

Glomerular disease sometimes develops rapidly after an infection in other parts of the body.

❖ **Acute post-streptococcal glomerulonephritis (PSGN)**

It can occur after an episode of strep throat or, in rare cases, impetigo (a skin infection). The Streptococcus bacteria do not attack the kidney directly, but an infection may stimulate the immune system to overproduce antibodies, which are circulated in the blood and finally deposited in the glomeruli, causing damage. PSGN can bring on sudden symptoms of swelling (edema), reduced urine output (oliguria), and blood in the urine (hematuria). Tests will show large amounts of protein in the urine and elevated levels of creatinine and urea nitrogen in the blood, thus indicating reduced kidney function. High blood pressure frequently accompanies reduced kidney function in this disease.

PSGN is most common in children between the ages of 3 and 7, although it can strike at any age, and it most often affects boys. It lasts only a brief time and usually allows the kidneys to recover. In a few cases, however, kidney damage may be permanent, requiring dialysis or transplantation to replace renal function.

❖ **HIV infection**

The virus that leads to acute immune deficiency syndrome (AIDS), can also cause glomerular disease. Between 5 and 10 percent of people with HIV experience kidney failure, even before developing full-blown AIDS. HIV-associated nephropathy usually begins with heavy proteinuria and progresses rapidly (within a year of detection) to total kidney failure. Researchers are looking for therapies that can slow down or reverse this rapid deterioration of renal function, but some possible solutions involving immune suppression are risky because of the patients' already compromised immune system.

- **Glomerulo sclerosis**

It is scarring (sclerosis) of the glomeruli. In several sclerotic conditions, a systemic disease like lupus or diabetes is responsible. Glomerulo sclerosis is caused by the activation of glomerular cells to produce scar material. This may be stimulated by molecules called growth factors, which may be made by glomerular cells themselves or may be brought to the glomerulus by the circulating blood that enters the glomerular filter.

- **Diabetic nephropathy**

High blood sugar from diabetes progressively damages the kidneys, eventually causing chronic kidney disease. Protein in the urine (Nephrotic syndrome) may also result. It is the leading cause of glomerular disease and of total kidney failure in the United States. Kidney disease is one of several problems caused by elevated levels of blood glucose, the central feature of diabetes. In addition to scarring the kidney, elevated glucose levels appear to increase the speed of blood flow into the kidney, putting a strain on the filtering glomeruli and raising blood pressure.

Diabetic nephropathy usually takes many years to develop. People with diabetes can slow down damage to their kidneys by controlling their blood glucose through healthy eating with moderate protein intake, physical activity, and medications. People with diabetes should also be careful to keep their blood pressure at a level below 140/90 mm Hg, if possible. Blood pressure medications called ACE inhibitors and ARBs are particularly effective at minimizing kidney damage and are now frequently prescribed to control blood pressure in patients with diabetes and in patients with many forms of kidney disease.

- **Focal segmental glomerulosclerosis (FSGS)**

It describes scarring in scattered regions of the kidney, typically limited to one part of the glomerulus and to a minority of glomeruli in the affected region. FSGS may result from a systemic disorder or it may develop as an idiopathic kidney disease, without a known cause. Proteinuria is the most common symptom of FSGS,

but, since proteinuria is associated with several other kidney conditions, the doctor cannot diagnose FSGS on the basis of proteinuria alone. Biopsy may confirm the presence of glomerular scarring if the tissue is taken from the affected section of the kidney. But finding the affected section is a matter of chance, especially early in the disease process, when lesions may be scattered.

Confirming a diagnosis of FSGS may require repeat kidney biopsies. Arriving at a diagnosis of idiopathic FSGS requires the identification of focal scarring and the elimination of possible systemic causes such as diabetes or an immune response to infection. Since idiopathic FSGS is, by definition, of unknown cause, it is difficult to treat. No universal remedy has been found, and most patients with FSGS progress to total kidney failure over 5 to 20 years. Some patients with an aggressive form of FSGS reach total kidney failure in 2 to 3 years. Treatments involving steroids or other immunosuppressive drugs appear to help some patients by decreasing proteinuria and improving kidney function. But these treatments are beneficial to only a minority of those in whom they are tried, and some patients experience even poorer kidney function as a result. ACE inhibitors and ARBs may also be used in FSGS to decrease proteinuria. Treatment should focus on controlling blood pressure and blood cholesterol levels, factors that may contribute to kidney scarring.

- **Membranous nephropathy**

Also called membranous glomerulopathy, is the second most common cause of the Nephrotic syndrome (proteinuria, edema, high cholesterol) in U.S. adults after diabetic nephropathy. Diagnosis of membranous nephropathy requires a kidney biopsy, which reveals unusual deposits of immunoglobulin G and complement C3, substances created by the body's immune system. Fully 75 percent of cases are idiopathic, which means that the cause of the disease is unknown. The remaining 25 percent of cases are the result of other diseases like systemic lupus erythematosus, hepatitis B or C infection, or some forms of cancer. Drug therapies involving penicillamine, gold, or captopril have also been associated with membranous nephropathy. About 20 to 40 percent of patients with membranous nephropathy progress, usually over decades, to total kidney failure, but most patients experience either complete remission or continued symptoms without progressive kidney failure. Doctors disagree about how aggressively to treat this condition, since about 20

percent of patients recover without treatment. ACE inhibitors and ARBs are generally used to reduce proteinuria. Additional medication to control high blood pressure and edema is frequently required. Some patients benefit from steroids, but this treatment does not work for everyone. Additional immunosuppressive medications are helpful for some patients with progressive disease.

- **Minimal change disease (MCD)**

It is the diagnosis given when a patient has the Nephrotic syndrome and the kidney biopsy reveals little or no change to the structure of glomeruli or surrounding tissues when examined by a light microscope. Tiny drops of a fatty substance called a lipid may be present, but no scarring has taken place within the kidney. MCD may occur at any age, but it is most common in childhood. A small percentage of patients with idiopathic Nephrotic syndrome do not respond to steroid therapy. For these patients, the doctor may recommend a low-sodium diet and prescribe a diuretic to control edema. The doctor may recommend the use of non steroidal anti-inflammatory drugs to reduce proteinuria. ACE inhibitors and ARBs have also been used to reduce proteinuria in patients with steroid-resistant MCD. These patients may respond to larger doses of steroids, more prolonged use of steroids, or steroids in combination with immunosuppressant drugs, such as chlorambucil, cyclophosphamide, or cyclosporine.

- **Renal failure**

Renal failure is any acute or chronic loss of kidney function and is the term used when some kidney function remains.

- **Acute Renal Failure**

It is the sudden worsening of the kidney functions. Dehydration, a blockage in the urinary tract, or kidney damage can cause acute renal failure, which is usually reversible. A few forms of glomerular disease cause very rapid deterioration of kidney function. For example, PSGN can cause severe symptoms (hematuria, proteinuria, edema) within 2 to 3 weeks after a sore throat or skin infection develops. The patient may temporarily require dialysis to replace renal function. This rapid loss

of kidney function is called acute renal failure (ARF). Although ARF can be life-threatening while it lasts, kidney function usually returns after the cause of the kidney failure has been treated. In many patients, ARF is not associated with any permanent damage. However, some patients may recover from ARF and subsequently develop CKD.

- **Chronic Kidney Disease**

A permanent partial loss of the functions of the kidney, Diabetes and high blood pressure are the most common causes. It is the most forms of glomerular disease develop gradually, often causing no symptoms for many years. CKD is the slow, gradual loss of kidney function. Some forms of CKD can be controlled or slowed down. For example, diabetic nephropathy can be delayed by tightly controlling blood glucose levels and using ACE inhibitors and ARBs to reduce proteinuria and control blood pressure. But CKD cannot be cured. Partial loss of renal function means that some portion of the patient's Nephrons has been scarred, and scarred Nephrons cannot be repaired. In many cases, CKD leads to total kidney failure.

- **End-stage renal disease (ESRD)**

It is the complete loss of kidney function, usually due to progressive chronic kidney disease. People with ESRD require regular dialysis for survival. The Total kidney failure, sometimes called end-stage renal disease (ESRD), indicates permanent loss of kidney function. Depending on the form of glomerular disease, renal function may be lost in a matter of days or weeks or may deteriorate slowly and gradually over the course of decades.

- **Total Kidney Failure**

To stay alive, a patient with total kidney failure must go on dialysis hemo-dialysis or peritoneal dialysis or receive a new kidney through transplantation. Patients with CKD who are approaching total kidney failure should learn as much about their treatment options as possible so they can make an informed decision

when the time comes. With the help of dialysis or transplantation, many people continue to lead full, productive lives after reaching total kidney failure.

2.1.6 SIGNS AND SYMPTOMS OF NEPHRON PATHOLOGY[21]

- **Edema:** Swelling caused by the accumulation of fluid in cells and tissues. In kidney failure, fluid may collect in the feet, hands, abdomen, or face.
- **Hematuria:** Blood in the urine. Blood may turn the urine pink or cola-colored.
- **Hypoproteinemia:** Reduced levels of protein in the blood.
- **Proteinuria:** Large amounts of protein in the urine, it may cause foamy urine.
- **Uremia:** Accumulation of urea and other wastes in the blood. These wastes, which become toxic in large amounts, are normally eliminated through urination.
- **Albuminuria:** Large amounts of protein in the urine
- **Reduced glomerular filtration rate:** Inefficient filtering of wastes from the blood
- General ill feeling and fatigue
- Generalized itching (pruritus) and dry skin
- Headaches
- Weight loss without trying to lose weight
- Appetite loss
- Nausea

Other symptoms like,

- Abnormally dark or light skin
- Bone pain
- Brain and nervous system symptoms
- Drowsiness and confusion
- Problems concentrating or thinking
- Numbness in the hands, feet, or other areas
- Muscle twitching or cramps
- Breath odour
- Easy bruising, bleeding, or blood in the stool

- Excessive thirst
- Frequent hiccups
- Low level of sexual interest and impotence
- Menstrual periods stop (amenorrhea)
- Sleep problems, such as insomnia, restless leg syndrome, and obstructive sleep apnea
- Swelling of the feet and hands (edema)
- Vomiting, typically in the morning

2.1.7 DISEASES AND CONDITIONS[21]

- **Autoimmune disease**

  It is a disease in which the body's own disease-fighting cells attack the body itself.

- **Hypertension**

  It is a condition that can cause kidney damage or be caused by kidney disease.

- **Idiopathic disease**

  It is a type of disease that occurs without a known cause.

- **Nephrotoxic**

  It is the damaging to the kidneys.

- **Sclerotic disease**

  It is a disease in which tissues become hardened or scarred.

- **Systemic disease**

  It is a disease that affects multiple parts of the body, often as a result of substances circulating in the blood.

2.2 NEPHROTOXICITY

Nephrotoxicity is toxicity in the kidneys. It is a poisonous effect of some substances, both toxic chemicals and medications, on renal function. There are varies forms, and some drugs may affect renal function in more than one way. Nephrotoxins are substances which displaying Nephrotoxicity. Nephrotoxicity should not be confused with the fact that some medications have a predominantly renal excreation and need their dose adjusted for the decreased renal function (example: heparin).[23]

Kidney failure is the one of the most common diseases in India. The world health organisation recognizes four major groups of renal failure according to the predominant involvement of corresponding morphologic component.

i. Glomouruler diseases,

ii. Tubular diseases,

iii. Interstitial diseases and

iv. Vascular diseases.

And also two major stages viz.

a) Acute renal failure – it is a syndrome charecterised by rapid onset of renal dysfunction, chiefly oliguria or anuria, and sudden increase in metabolic waste product in the blood and secondly

b) Chronic renal failure – it is a syndrome charecterised by progressive and irreversible deterioration of renal function due to slow destruction of renal parenchyma eventually terminating in death.

Many plants have been used for the treatment of kidney failure in traditional system of medicine throughout the world. Indeed along with the dietary measures, plant preparation formed the basis of the treatment of the disease until the introduction of allopathic medicine. Ethnomedicinal plants can be used to help forestall the need of dialysis by treating the causes and effect of renal failure, as well as reducing the many adverse effect of dialysis.[24]

There are few chemical agents to treat acute renal failure. Studies reveal back synthetic Nephroprotective agents have adverse effect besides reduce Nephrotoxicity, various environmental toxical and clinically useful drugs, acetaminophen, gentamycin and Cisplatin, can cause severe organ toxicities through the metabolic activation to highly reactive free radicals.[25]

The term acute renal failure (ARF) is currently substituted by acute kidney injury (AKI). Acute kidney injury is a reversible condition in which there is a sudden decline in renal function, manifested by hourly/ daily/ weekly elevation in serum creatinine and blood urea nitrogen (BUN).[26]

Different organisation such as acute dialysis quality initiative (ADQI), acute renal injury network (AKIN) and kidney disease international global outcome (KDIGO) have proved different definition for acute kidney injury. Among the definition of acute kidney injury (AKI) the most acceptable one is kidney disease international global outcome (KDIGO).[27]

Acute kidney injury is may be any of the following,[28]

- "Increase in serum creatnine (SCr) by $\geq$ 0.3mg/dl ($\geq$ 26.5µmol/l) within 48 hours" or
- "Increase in serum creatinine to $\geq$ 1.5 times baseline, which is known or presumed to have occurred within the prior 7 days" or
- "Urine volume < 0.5 ml/kg/h for 6 hours".

The incidence of acute kidney injury in the community is 2147 and 4085 per million populations per year (PMP) in developing and developed nations.[29, 30] Recent reports in the developed world indicate that acute kidney injury is seen in 3.2 – 9.6 % of hospital admissions with overall mortality of 20%-50% in ICU patients.[31, 32] Acute kidney injury demanding renal replacement therapy is 5-6% with a high in hospital mortality rate of 60%. It is estimated that nearly 2 million people die of acute kidney injury every year globally.[33, 34] Those who survive acute kidney injury are at a greater risk for later development of chronic kidney disease (CKD).

The cause of acute kidney injury could be pre-renal, interensic renal and post renal ones.

Pre-renal failure- It is the commonest form of acute renal failure, it is due to decrease in renal blood flow primarily as a result of hypovolemia. It is reversible if the cause of decreased renal blood flow can be identified and rectified before kidney damage occures. Pre-renal causes account for 40-70% of all acute kidney injury patients.[35]

Interensic renal causes of AKI include diseases that affect the renal parenchyma which can be divided based on the compartment of the kidney that is affected like tubular injury, tubuloninterstitial disease, disease of the renal microcirculation and glomeruli and diseases of larger renal vessels.

Post renal causes of AKI are diseases associated with urinary tract obstruction which account for 5% of renal failures.[36]

2.2.1 DRUG INDUCED NEPHROTOXICITY IN HUMAN

1. AMINOGLYCOSIDE INDUCED NEPHROTOXICITY

Aminoglycosides preferentially affect the proximal tubular cells. These agents are freely filtered by the glomeruli and quickly taken up by the proximal tubular epithelial cells, where they are incorporated into lysosomes after first interacting with phospholipids on the brush border membranes. They exert their main toxic effect within the tubular cell by altering phospholipid metabolism. In addition to their direct effect on cells, aminoglycosides cause renal vasoconstriction.

The two critical factors in the development of acute kidney injury (AKI) secondary to aminoglycoside Nephrotoxicity are dosing and duration of therapy. Aminoglycoside uptake by the tubules is a saturable phenomenon, so uptake is limited after a single dose. Thus, a single daily large dose is preferable to 3 doses per day. One dose per day presumably causes less accumulation in the tubular cells once the saturation point is reached.[37, 38]

2. AMPHOTERICIN B INDUCED NEPHROTOXICITY

Amphotericin B binds to sterols in cell membranes, thereby creating pores that compromise membrane integrity and increase membrane permeability. It binds not only to ergosterol in fungal cell walls but also to cholesterol in human cell membranes; this is what accounts for its Nephrotoxicity.

Characteristic electrolyte abnormalities include wasting of potassium and magnesium secondary to increased permeability of the cell membranes. The back-leak of hydrogen ions in the collecting duct leads to distal renal tubular acidosis (dRTA).[39, 40]

Lipid-based preparations of amphotericin B decrease but do not eliminate the Nephrotoxicity compared with traditional amphotericin B.[41] This may be due to a direct nephrotoxic effect of the conventional preparation.

3. CONTRAST-INDUCED NEPHROPATHY

Although the pathogenesis of contrast-induced nephropathy (CIN) remains incompletely understood, it is most likely the result of renal vasoconstriction and direct renal tubular epithelial cell toxicity. Current theories regarding CIN toxicity include a combination of direct cytotoxicity with postischemic reperfusion injury resulting in oxygen free radical production leading to endothelial damage.[42, 43]

4. CALCINEURIN INHIBITOR INDUCED NEPHROTOXICITY

Cyclosporine and tacrolimus cause acute kidney injury (AKI) by inducing afferent and efferent arteriolar vasoconstriction. Persistent injury can lead to interstitial fibrosis. Tacrolimus has been shown to cause thrombotic microangiopathy as a result of endothelial injury.[44, 45]

5. CISPLATIN INDUCED NEPHROTOXICITY

Cisplatin usually affects the proximal tubules primarily with some secondary effect on the glomeruli and distal tubules. Cisplatin is excreted primarily in the urine, resulting in concentrated drug levels, which encourage uptake into the cells by passive diffusion or active uptake. Cisplatin is stable in the blood stream but

becomes hydrolyzed in the chloride-poor cellular environment. It is the hydrolyzed metabolite that binds DNA, RNA, proteins, and phospholipids, causing cytotoxicity.[46]

MECHANISM OF CISPLATIN NEPHROTOXICITY[47]

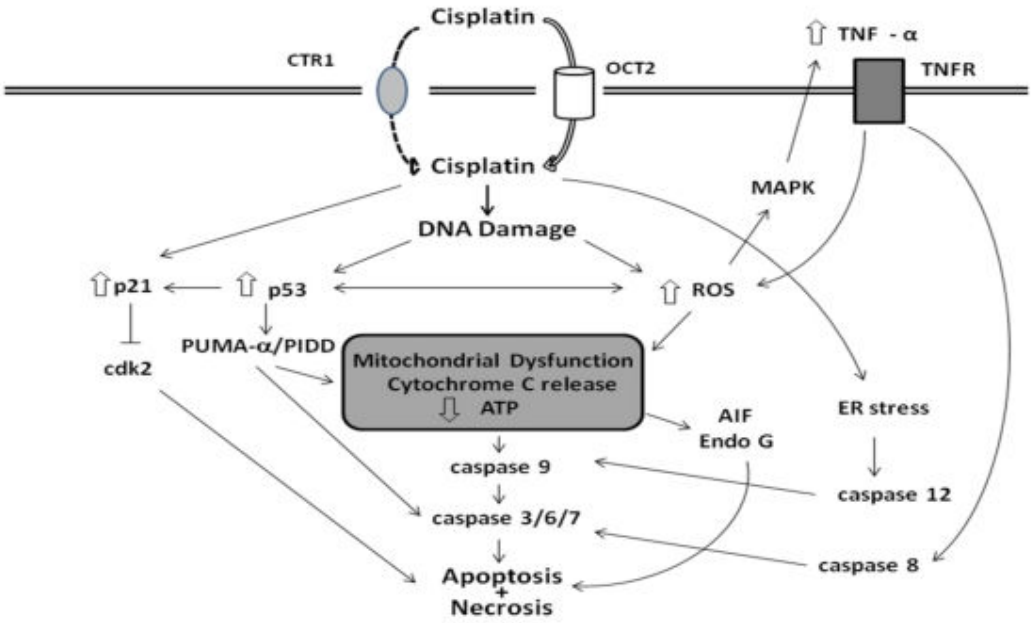


Fig.3: Pathways of Cisplatin-induced epithelial cell death

Cisplatin enters renal epithelial cells via the OCT2 and, to a lesser extent, Ctr1 transporters. Cisplatin causes damage to nuclear and mitochondrial DNA and production of reactive oxygen species (ROS) which lead to activation of both mitochondrial and non-mitochondrial pathways of apoptosis and necrosis.

Mitochondrial energetic are also disrupted by Cisplatin and may contribute to Nephrotoxicity. Fatty acids are the major source of energy for the proximal tubule, the primary site of Cisplatin kidney injury. Cisplatin inhibits fatty acid oxidation in rat kidney and in proximal tubule cells in culture through a reduction in PPAR-α mediated expression of genes involved in cellular fatty acid utilization.

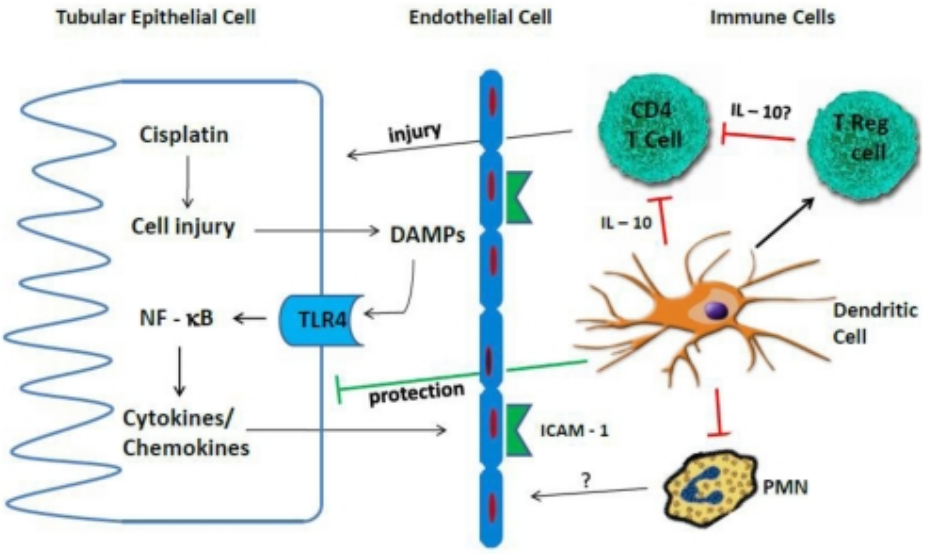


Fig.4: Pathways of Cisplatin-induced inflammation in renal cell

Cisplatin-induced injury to renal epithelial cells causes release of DAMPs, which activate TLR4. Activation of TLR4 results in the production of a variety of chemokines and cytokines, including TNF-α

. These chemokines and cytokines up regulate adhesion molecules and attract inflammatory cells, such as neutrophils and T cells, into the region of injury.

6. IFOSFAMIDE INDUCED NEPHROTOXICITY

Ifosfamide is a known analog of cyclophosphamide. Although cyclophosphamide is not nephrotoxic, ifosfamide, by virtue of its metabolite chloroacetaldehyde, is toxic to the tubular cells, with preferential involvement of the proximal tubule leading to Fanconi syndrome.[48, 49]

7. FOSCARNET INDUCED NEPHROTOXICITY

Foscarnet, which is used to treat resistant cytomegalovirus (CMV) infections, causes acute interstitial nephritis and intra-tubular crystal formation. In addition to crystal formation, which can be made up of calcium salts or sodium salts, chelation of calcium by foscarnet leads to hypocalcemia.[50, 51]

8. CRYSTAL-FORMING DRUG INDUCED NEPHROTOXICITY

Sulfa drugs, acyclovir, methotrexate, ethylene glycol, and protease inhibitors like indinavir cause acute kidney injury (AKI) by tubular obstruction due to crystal formation in the tubular urine.

Acyclovir may lead to the formation of intratubular crystals, which appear as birefringent needle-shaped crystals and can elicit an acute interstitial nephritis.[52, 53]

9. RHABDOMYOLYSIS INDUCED NEPHROTOXICITY

Rhabdomyolysis refers to the breakdown of skeletal muscle fibers, which leads to the release of potentially nephrotoxic intracellular contents into the circulation. Acute kidney injury (AKI) develops in this setting via the following 3 mechanisms:

- Renal vasoconstriction
- Heme-mediated proximal tubular cell toxicity
- Intratubular cast formation

Heme proteins are believed to be involved in the generation of reactive oxygen species (ROS), which are known to cause tubular injury through peroxidation of membrane lipids and intracellular enzymes.[54]

10. MULTIPLE MYELOMA INDUCED NEPHROTOXICITY

Multiple myeloma causes renal failure by several mechanisms. The extra protein can be deposited in the kidney as amyloidosis or monoclonal immunoglobulin deposition disease affecting the glomeruli. Light-chain cast nephropathy occurs when light chains become concentrated in the tubular lumen. Plasma cells can infiltrate the kidney directly, causing kidney dysfunction. Hyper-calcemia can independently cause renal vasoconstriction. Volume depletion and medications used to treat multiple myeloma can also contribute to renal disease.[55, 56]

2.2.2 Kidney function test[57]

There are a number of urine tests that can be used to assess kidney function. A small, randomly collected urine sample is examined physically for colour, odour, appearance, and concentration (specific gravity); chemically, for substances such a protein, glucose, and *pH* (acidity/alkalinity).

- **Glomerular function test:**

  The entire clearance tests like Inulin, Creatinine, and Urea.

- **Tubular function test:**

  Urine concentration or urine acidification test is used to determine tubular function.

- **Analysis of blood serum:**

  - Serum Creatinine levels
  - Blood urea nitrogen (BUN)
  - Serum protein
  - Calcium
  - Magnesium
  - Phosphorus and
  - Uric acid

- **Analysis of urine:**

  - Analysis of urea
  - Uric acid
  - Calcium
  - Oxalates
  - Magnesium
  - Phosphorus and
  - Citrate

Causes of chronic kidney disease may be seen on

- Abdominal CT scan
- Abdominal MRI
- Abdominal ultrasound and Renal scan

2.2.3 TREATMENT OF NEPHROTOXICITY[21]

The treatment plan for Nephrotoxicity may include:

- **Biopsy**

It is a procedure in which a needle is used to obtain small pieces of tissue from an organ for examination under different types of microscopes, each of which shows a different aspect of the tissue.

- **Dialysis**

It is the medical treatment that removes wastes and extra fluid from the blood after the kidneys have stopped working.

- **Immunosuppressant**

It is the medicine given to block the body's immune system.

- **Plasmapheresis**

It is a medical treatment in which the blood is treated outside the body to remove harmful antibodies, and then returned to the patient.

2.2.4 PHARMACOLOGICAL THERAPY[58]

Although medicine cannot reverse chronic kidney disease, it is often used to help treat symptoms and complications and to slow further kidney damage.

Medicines to treat high blood pressure

Most people who have chronic kidney disease have problems with high blood pressure at some time during their disease. Medicines that lower blood pressure help to keep it in a target range and stop any more kidney damage.

Common blood pressure medicines include:

- **ACE inhibitors**
  - Captopril
  - Zofenopril
  - Enalapril
  - Ramipril
- **Angiotensin II receptor blockers (ARBs)**
  - Losartan
  - Vasartan
  - Candesartan
  - Telmisartan
  - Olmesartan
- **Beta-blockers**
  - Propranolol
  - Metoprolol
  - Atenolol
  - Labetalol
- **Calcium channel blockers**
  - Verapamil
  - Diltiazem
  - Nifedipine
  - Felodipine
  - Amlodipine
  - Benidipine

- **Direct renin inhibitors**
- **Diuretics**
  - Furosemide
  - Spironolactone
  - Amiloride
  - Indapamide
  - Chlorthalidone
  - hydrochlorothiazide
- **Vasodilators**
  - Minoxidil
  - Diazoxide
  - Hydralazine
  - Sodium nitroprusside

Medicines to treat symptoms and complications of chronic kidney disease

Medicines may be used to treat symptoms and complications of chronic kidney disease. These medicines include:

- **Erythropoietin (rhEPO) therapy** and **iron replacement therapy** (iron pills or intravenous iron) for anemia.

Medicines for electrolyte imbalances

- **Diuretics** to treat fluid buildup caused by chronic kidney disease.
- **ACE inhibitors and ARBs**. These may be used if you have protein in your urine (proteinuria) or have heart failure.

Regular blood tests are required to make sure that these medicines don't raise potassium levels (hyperkalemia) or make kidney function worse.

Medicines used during dialysis

- Both **erythropoietin (rhEPO) therapy and iron replacement therapy** may also be used during dialysis to treat anemia, which often develops in advanced chronic kidney disease.
- **Erythropoietin (rhEPO)** stimulates the production of new red blood cells and may decrease the need for blood transfusions. This therapy may also be started before dialysis is needed, when anemia is severe and causing symptoms.
- **Iron therapy** can help increase levels of iron in the body when rhEPO therapy alone is not effective.
- **Vitamin D** helps keep bones strong and healthy.

2.2.5 AYURVEDIC TREATMENTS OF NEPHROTOXICITY[59]

The Ayurvedic treatment of chronic renal failure is based on three principles:

(i) Treating the damaged kidneys

(ii) Treating the body tissues (dhatus) which make up the kidneys and

(iii) Treating the known causes.

2.2.5.1 TREATING THE DAMAGED KIDNEYS

The damage done to the kidneys can be repaired using medicines like

a) Punarnavadi Guggulu

b) Gokshuradi Guggulu

c) Gomutra Haritaki

d) Chandraprabha Vati and

e) Punarnavadi Qadha (decoction).

Herbal medicines useful in this condition are:

i. Punarnava (Boerhaavia diffusa)

ii. Gokshur (Tribulus terrestris)

iii. Haritaki (Terminalia chebula)

iv. Neem (Azadirachta indica)

v. Daruharidra (Berberis aristata) and

vi. Patol (Tricosanthe dioica).

2.2.5.2 TREATING THE BODY TISSUES (DHATUS) WHICH MAKE UP THE KIDNEYS

According to Ayurveda, the kidneys are made up of the "Rakta" and "Meda" dhatus. Treating these two dhatus is also an effective way to treat the kidneys.

Medicines used are:

- Patol
- Saariva
- Patha (Cissampelos pareira)
- Musta (Cyperus rotundus)
- Kutki (Picrorrhiza kurroa)
- Chirayta (Swertia chirata)
- Guduchi (Tinospora cordifolia)
- Chandan (Santalum album) and
- Shunthi (Zinziber officinalis).

2.2.5.3 TREATING THE KNOWN CAUSES

Lastly, the known cause of chronic renal failure is treated using medicines which also act upon the kidneys. Vascular (related to the blood vessels) diseases like renal artery stenosis and inflammation of the artery walls (vasculitis) can be treated using medicines like

i. Arogya Vardhini

ii. Tapyadi Loha

iii. Mahamanjishthadi Qadha

iv. Kamdudha Vati

v. Manjishtha (Rubia cordifolia)

vi. Bhrungraj (Eclipta alba)

vii. Saariva, Kutki and

viii. Sarpagandha (Rauwolfia serpentina).

Primary glomerular diseases like membranous nephropathy and glomerlonephritis can be treated using

- Punarnava
- Gokshur
- Saariva and
- Manjishtha.

Secondary glomerular disease resulting from diabetes, systemic lupus erythematosus, rheumatoid arthritis etc. can be treated accordingly, using the medicines appropriate for those diseases. Similarly, suitable Ayurvedic medicines can be given for other causes like polycystic kidneys, prostate enlargement and neurogenic bladder.

2.2.5.4 ADVANTAGES OF AYURVEDIC MEDICATIONS

The advantage of using Ayurvedic medicines in chronic renal failure is that in most patients, the kidney damage can be either partly or fully reversed, the frequency of dialysis can be reduced, and the increased risk of death from cardiovascular diseases can be significantly reduced. Thus, Ayurvedic medicines have the potential for an important therapeutic contribution in all the stages of this condition.

2.3 HERBAL PLANTS USED IN NEPHROTOXICITY

Herbal Plants have the ability to synthesize a wide variety of chemical compounds that are used to perform important biological functions and to defend against attack from predators such as insects, fungi and herbivorous mammals. Many of these photochemical have beneficial effects on long-term health when consumed by humans and can be used effectively treat human disease.

Pharmacologists, microbiologists, botanists and natural-product chemists are combing the earth for phytochemicals and leads that could be developed for the treatment of various diseases. In fact, according to the World Health Organization, approximately 25% of modern drugs used in the United States have been derived from plants.

Numbers of medicinal plants show Nephroprotective activity and thereby play a vital role in the prevention and treatment of nephrtoxicity.

Table-1 List of plants used Nephroprotective activity

Sl no.	Botanical name	Family	Part used	Extract used	Method off inducing nephro-toxicity	Reference
1	*Aerva lanata*	*Amaranthaceae*	Whole plant	Aqueous Extract	Gentamicin	60
2	*Azima tetracantha*	*Salvadoraceae*	Root	Ethanolic extract	Glycerol	61
3	*Barringtunia acutangula*	*Barringtoniaceae*	Whole plant	Methanol-dichloromethane	Gentamicin	62
4	*Boerhaavia diffusa*	*Nyctaginaceae*	Root	Ethanolic Extract	Ethylene glycol	63

5	*Croton zambesicus*	*Euphorbiaceae*	Root	Ethanolic extract	Gentamicin	64
6	*Indigefera tinctoria*	*Fabaceae*	Root and leaves	Aqueous extract	Cisplatin	65
7	*Oroxylum indicum*	*Bignoniaceae*	Whole plant	Methanol-dichloromethane	Gentamicin	62
8	*Solanum xanthocarpum*	*Solananaceae*	Fruit	Ethanolic extract	Gentamycin	66
9	*Tamarindus indica*	*Fabaceae*	Leaves	Methanolic extract	Gentamicin	67
10	*Tribulus terrestris*	*Zygophyllaceae*	Fruit	Dried powder	Gentamicin	63
11	*Vitex negundo*	*Verbanaceae*	Root	Methanol-dichloromethane	Gentamicin	62
12	*Volveriella volvacea*	*Pluteaceae*	Cultured myceliu m	Aqueous-ethanolic extract	Doxorubicin	68

2.4 EXPERIMENTAL DESIGN

A number of models are used for the study of Nephroprotective activity. An appropriate experimental nephrotoxic model is of importance for studying the pathogenesis of Nephrotoxicity, evaluating the relative importance of various nephrotoxic factors and assessing the efficacy of different drugs in nephroprotectivity.[69]

Sixteen models are used for inducing Nephrotoxicity in rats, they are:

1) **Glycerol-induced acute renal failure.**
2) **Ischemia-reperfusion-induced acute renal failure.**
3) **Gentamicin-induced acute renal failure.**
4) **Cisplatin-induced acute renal failure.**
5) **Radiocontrast media-induced acute renal failure.**
6) **NSAID-induced acute renal failure.**
7) **Osmosis-induced acute renal failure.**
8) **Ifosfamide-induced acute renal failure.**
9) **Uranium-induced acute renal failure.**
10) **Mercuric chloride-induced acute renal failure.**
11) **Potassium dichromate-induced acute renal failure.**
12) **Folic acid-induced acute renal failure.**
13) **Ferric-nitrilotriacetate-induced acute renal failure.**
14) **S-(1,2-dichlorovinyl)-L-cysteine-induced acute renal failure.**
15) **Sepsis-induced acute renal failure.**
16) **Bipyridyls-induced acute renal failure.**

1. Glycerol-induced acute renal failure

Glycerol-induced ARF is characterized by myoglobinuria, tubular necrosis[70] and enhanced renal vasoconstriction. The pathogenic mechanisms involved in glycerol-induced renal failure include ischemic injury, tubular Nephrotoxicity caused by myoglobin, and the renal actions of cytokines released after rhabdomylosis.[71,72] The large numbers of disorders known to cause rhabdomyolysis include intrinsic muscle dysfunction (including trauma, burns, intrinsic muscle disease, and excessive physical exertion), metabolic disorders, hypoxia, drugs, toxins, infections, temperature extremes and idiopathic disorders.[73] Complications associated with rhabdomyolysis include disseminated intravascular coagulation, hyper kalemia and other metabolic imbalances, ARF and acute cardiomyopathy. In general, about 10–40% of cases with rhabdomyolysis develop ARF and it accounts for 2–15% of all cases of ARF. The model for studying this form of ARF is obtained in the rat by intramuscular injection of glycerol.[74] There is enhanced generation of hydrogen peroxide in renal cortex in rats with glycerol-induced acute renal failure. A standard method of inducing renal failure is by intramuscular administration of 50% glycerol, v/v (8 ml/kg, im).[75] The required amount of glycerol is administered as a deep im injection equally distributed to both hind legs. Rats are deprived of food and water for 24 h before glycerol administration after which they were sacrificed for kidney function evaluation.[76] Also, Vlahovic et al. induced ARF by administration of glycerol (50% v/v in saline) im at a dose of 10 ml/kg. Injection volumes were divided equally between two hind limbs. The rats were dehydrated 18 h prior induction of myoglobinuric renal injury and sacrificed 48 h after injection of hypertonic glycerol without any restriction of diet or water.[77] Intramuscular injection of glycerol in the rabbit induces a model of ARF at a dose of 10 mg/kg that resembles the ARF caused by massive release of myoglobin in crush syndrome in humans.[78, 79] An intramuscular administration of single dose of 8 ml/kg of glycerol is the most appropriate animal model that clinically mimics the rhabdomyolysis-induced renal failure in humans.

2. Ischemia-reperfusion-induced acute renal failure

Under the circumstances such as ischemia and nephrotoxins, ARF is characterized by “acute tubular necrosis” with flattened epithelia and tubular dilation

and cast formation. In these conditions, the tubular damage and altered glomerular hemodynamics may coexist or even lead to each other.[80] Although the detailed cellular and molecular mechanisms of cell injury and the subsequent recovery are not entirely known, yet, data from the previous studies have indicated that ARF may result from the necrosis and apoptosis of renal epithelial cells.[81, 82, 83] In the kidney, ischemia reperfusion injury is associated with cell death of tubular epithelial cells, localized in the stripe between the cortex and medulla; via necrosis or apoptosis that in-turn depends on the severity of the ischemic insult. Experimentally, ARF is induced by clamping the left renal artery for 1 h followed by reperfusion in anesthetized uninephrectomized dogs and renal failure is noted to develop within 3 h.[84] Bhalodia et al. have reported the development of renal failure in rats within 24 h by clamping of both the kidneys for 60 min followed by 24 h of reperfusion.[85, 86] The development of I/S-induced ARF in rats has also been demonstrated by unilateral left renal artery clamping using a small non traumatic vascular clamp for 45 min followed by reperfusion for 24 h.[87,88] Foglieni et al. have reported the development of renal failure in rats by clamping both right renal artery and vein for 60 min with a microsurgical clamp followed by reperfusion for 60 min.[89] Baker et al. have reported the development of renal failure in pigs by occluding infra-renal aorta with a standard angled arterial cross-clamp (palpation of distal aorta to confirm total aortic occlusion) for 150 min followed by reperfusion for 180 min.[90] Matthijsen et al. have reported the development of I/R-induced renal failure in mice by applying a non traumatic vascular clamp to the left renal pedicle for 40 min after 1.0 cm long midline abdominal incision to induce ischemia followed by reperfusion for 24 h.[91] Similarly, Susa et al. have reported the development of renal failure in mice by occluding left renal artery by an atraumatic microvascular clamp to induce ischemia lasting for 25–37 min with reperfusion of 24 h.[92] The ischemia of 45 min followed by 24 h reperfusion is more suitable and commonly used animal model to simulate the hemodynamic changes-induced alteration in renal function in humans.

3. Gentamicin-induced acute renal failure

In humans, gentamicin has been used for the treatment of life threatening Gram negative infections. Clinically, the high dose of gentamicin (2.5 mg/kg, im every 12 h for 7 days) has been shown to produce Nephrotoxicity.[93] It has been reported that 30% of patients treated with gentamicin for more than 7 days show

signs of Nephrotoxicity.[94] Gentamicin Nephrotoxicity is one of the most common causes of ARF and promotes both increased morbidity and greater health care costs. The clinical trial reports of elder patients have documented that aminoglycosides levels above 2.5µg/ml possess the major risk factors for aminoglycoside-associated Nephrotoxicity.[95, 96] The mechanism of renal failure is that the polycationic aminoglycoside gentamicin is prefrentially uptaken by proximal tubular cells of the Nephron by binding to negatively charged phospholipids on the brush border and is then quickly transferred to the transmembrane protein megalin.[97] After internalization via endocytosis, the aminoglycoside is transported to the lysosome and tightly binds to acidic phospholipids in the lipid bilayer, causing reduced phospholipase activity and production of phospholipid metabolites. The ability of gentamicin to alter mitochondrial respiration has been well documented in reports of both in vitro and in vivo studies.[98] Other factors that contribute to the pathogenesis of gentamicin Nephrotoxicity include generation of superoxide anion and hydroxyl radicals, alteration of anti-oxidant defense systems, depletion of reduced glutathione, Na^+-K^+-ATPase inhibition, opening of mitochondrial permeability transition pore and activation of renin-angiotensin system.[99, 100, 101, 102] Different methods have been employed to induce renal failure in rats that include ip administration of gentamicin sulfate at a dose of 100 mg/kg/day (in 0.9% NaCl) for 5–8 days and assessment of renal failure assessed 24 h after the last gentamicin injection.[103] Xie et al. reported the development of ARF in rats by administration of relatively higher dose of gentamicin sulfate at the dose of 150 mg/kg, sc route for five days.[104] On the other hand, in another variation, the development of ARF in rats has been shown by administrating gentamicin at a dose of 200 mg/kg twice daily for four consecutive days.[105] Volpini et al. have reported the development of ARF in rats by administration of gentamicin at a dose of 40 mg/kg, im, twice a day for nine days,[106] while Bledsoe et al. reported the development of ARF in rats by administration of gentamicin at a dose of 80 mg/kg, sc for ten days with the assessment of renal failure on the eleventh day.[107] The administration of gentamicin 100 mg/kg, ip, for 5 consecutive days inducing renal dysfunction is more commonly used model and closely mimics the antibiotic-induced changes in renal function in clinical setup.

4. Cisplatin-induced acute renal failure

Cisplatin [cis-diaminedichloroplatinum(II)], an anticancer drug, is broadly used for the therapy of cancers such as ovarian, head and neck carcinomas, and germ cell tumors. Nephrotoxicity is frequent and is the major limitation in Cisplatin-based chemotherapy. In humans, high dose of Cisplatin (75 mg/m2) has been used as baseline chemotherapeutic agent for the management of lung cancer. However, at this dose significant kidney damage has been seen in patients. The patients are administered saline infusion prior to and following Cisplatin (total of 3.5–4.0 liters during 3–4 h) to prevent Nephrotoxicity. The previous clinical studies had also reported that Cisplatin in a dosage of 20 mg/m2/day for 5 days causes significant changes in serum creatinine, creatinine clearance and 2.4 fold higher concentration of urine N-acetyl-b-D-glucosaminidase (an indicator of tubular damage) levels.[108] There are several mechanisms that contribute to renal dysfunction following exposure to Cisplatin that include direct tubular toxicity in the form of apoptosis and necrosis that is mediated through inflammation, reactive oxygen species (ROS), calcium overload, phospholipase activation, depletion of reduced glutathione, inhibition of mitochondrial respiratory chain function, induction of apoptosis, opening of mitochondrial permeability transition pore (MPTP) and ATP depletion.[109,110,111,112] Izuwa et al. have reported that administration of 5 ml/kg Cisplatin (0.1% of saline solution) in the abdominal cavity is associated with development of ARF in rats within 72 h of administration,[113] while Roncal et al. reported the development of renal failure with the same dose of Cisplatin after five days of drug injection.[114] Other reports have documented the development of renal failure with a single ip dose of 6 mg/kg,[115] 20 mg/kg and 30 mg/kg Cisplatin in rats within 72 h.[116,117] The ARF model has also been developed in mice by injecting a single dose of Cisplatin 16 mg/kg, ip and renal dys-functioning has been observed after 72 h of injection.[118] On the other hand, Lu et al. have reported the induction of ARF in mice by injecting Cisplatin at a dose of 30 mg/kg, ip.[119] Other research groups have also developed Cisplatin-induced renal failure model in mice by varying the dose of Cisplatin that include 12 mg/kg, ip;[120] 18 mg/kg, ip;[121] 40 mg/kg, ip.[122]

5. Radiocontrast media-induced acute renal failure

Clinically, radiocontrast media are very commonly used in radiology particularly for cardiac catheterization. Radiocontrast-induced nephropathy is a frequent clinical problem and is a major cause of acute renal failure.[123] Patients administered with radiocontrast media have been reported to exhibit an increased frequency of clinical adverse events including permanent impairment of renal function, longer hospital stay and increased mortality rate. The incidence of radiocontrast nephropathy approaches 30–50% in patients with volume depletion, congestive heart failure, preexisting renal failure, or diabetes mellitus.[124] In patients, isoosmolar radiocontrast (86% of iodixanol) and low-osmolar radiocontrast agent (14% of iohexol) induce acute renal failure.[125] The pathogenesis of radiocontrast nephropathy appears to be multifactorial and includes a deleterious reduction of renal arteriolar blood flow and glomerular filtration rate as well as the direct renal tubular toxicity caused by the radiocontrast agents.[126] The pathophysiology of toxic renal injury caused by radiocontrast media involves changes in generation of free radicals, inflammatory mediators, alteration of anti-oxidant defense systems and development of apoptosis.[127] Different research groups have employed different contrast media to develop renal failure models in animals. Diatrizoate is a water-soluble organic iodide contrast medium (1-deoxy-1-(methylamino)-D-glucitol-3,5-diacetamido-2,4,6-triiodobenzoate).[128] In the pure form, it contains 59.87% of organically bound iodine and 50% (w/v) solution contains 300 mg I/ml. It has an osmolality of 1550 mosm/kg, and is hypertonic to blood. Erley et al. have reported the development of ARF in rats by administration of sodium ditriazoate at a dose of 2 ml/kg into the jugular vein over a period of 2 min. After the injection, three clearance periods are performed each lasting 30 min, in which urine and blood sampling is done to assess the renal failure.[129] Yen et al. have reported the development of ARF in rats within 1 h by administration of 10 ml/kg of ditriazoate with an iodine load of 3700 mg/kg via the tail vein[130] while Colbay et al. reported the development of ARF in rats within 24 h by iv injection of ditriazoate (7 ml/kg) over a period of 5 min.[131]

Iohexol is commonly used as a non-ionic X-ray contrast media agent[132] and for the measurement of GFR. Iohexol does not bind to serum proteins and is 100% filtered through glomerulus, with no indications of tubular secretion or

reabsorption. Touati et al. have reported the development of renal failure in rats, in which rats were uninephrectomized and six days later, the aorta was clamped above the renal artery and a low-osmolar contrast media (CM), ioxaglate, was injected (1 ml/min; 3 min) via an aortic puncture in the single remaining kidney. The parameters to assess the renal failure were determined 24 and 48 h after CM administration.[133] Lee et al. have reported the development of renal failure in mice within 24 h by ip administration of iohexol (350 mg iodine/ml, 1.5–3 g iodine/kg). Kwak et al. have reported the development of renal failure in rats by administering three doses of the contrast medium named Ultravist via iv route: low dose (CT: 0.5 ml/kg = 0.15 g iodine/kg), standard (CT: 2 ml/kg = 0.6 g iodine/kg), and high-dose (CT: 8 ml/kg = 2.4 g iodine/kg). The blood sampling was done 48 h after the contrast injection to assess the renal failure.[134] Agmon et al. have reported the development of renal failure in rats by injecting sodium iothalamate (80%) through the arterial cannula over 2–3 min, at the dosage of 6 ml/kg. The blood samples were withdrawn 24 h after the contrast medium injection to assess the renal impairment.[135] Single dose administration of diatrizoate 7–10 ml/kg induced renal failure is more commonly used animal model to clinically simulate radiocontrast media-induced renal failure at the time of cardiac catheterization in patients.

6. NSAID-induced acute renal failure

Acetaminophen-induced acute renal failure

Acetaminophen is most widely used in the world as an analgesic and antipyretic drug that is safe at therapeutic dosages. However, it is also known to cause hepatic necrosis and renal failure in humans[136] and animals[137] in overdoses. In human, acetaminophen represents a growing cause of renal failure in current medical practice. Acetaminophen-induced renal insufficiency is consistent with acute tubular necrosis, an increase in the plasma creatinine level and a decrease in the GFR. The cumulative doses of acetaminophen and aspirin have been documented to induce the renal failure at the dose of 100–499 g and 500–2,999 g or ≥ 3,000 g, respectively, in patients.[138] Oxidative stress is reported to play a role in the pathogenesis of acetaminophen-induced renal damage whose metabolism occurs via cytochrome-P (CYP) 450 enzymes in both the liver and the kidneys. In renal tissues, prostaglandin synthetase and N-deacetylase enzymes play a key role in the

formation of free radicals and their metabolites. At higher doses, acetaminophen is shunted through these pathways leading to the increased production of reactive oxygen/nitrogen metabolites, gradual GSH depletion, formation of lipid peroxidative products leading to cell death and renal failure.[139, 140, 141] Palani et al. reported the development of ARF in rats within 24 h by administrating a single dose of acetaminophen (750 mg/kg, p.o).[142] Adeneye et al. and Cekmen et al. have reported the development of ARF within 24 h in rats by administration of a single dose of acetaminophen 800 mg/kg, ip, which was dissolved in normal saline.[143, 144] Recently, Kheradpezhouh et al. have reported that ARF may be induced in rats within 18–24 h by ip administration of a single dose of acetaminophen (700 mg/kg), dissolved in propylene glycol and distilled water (50:50).[145] Acetaminophen is also used to induce ARF in mice, as Li et al. have reported the development of ARF within 16 h in mice by administration of a single nephrotoxic dose of acetaminophen (600 mg/kg, dissolved in saline, 25 ml/kg, ip).[146] In another study, Chen et al. have reported the development of ARF within 4 h in mice with different age groups, i.e., young ones with age of 3–31 month and old ones with age of 30–31 months, by administration of the same dose of acetaminophen (375 mg/kg, ip) dissolved in ethanol: propylene glycol (1:4).[147] Single dose administration of acetaminophen (600–750 mg/kg) induced renal failure in rodents closely related to renal dysfunction due to overdose of acetaminophen in humans.

Diclofenac sodium-induced acute renal failure

Non-steroidal anti-inflammatory drugs (NSAIDs) are the most common prescription medicines and diclofenac is widely used NSAID for the management of pain and inflammation associated with arthritis. Unfortunately, one of the main side effects of NSAIDs administration is renal function damage.[148] Therefore, the research has been directed for exploring non-steroidal analgesics that do not exhibit the typical side effects associated with NSAIDS including renal failure.[149] In human, diclofenac has been widely used NSAID for the management of pain and inflammation associated with arthritis.[150] A clinical trial report has documented that diclofenac (75 mg/day for six months) induces severe renal injury.[151] NSAIDs mediated abrogation of prostaglandin synthesis and resultant renal ischemia is the major mechanism and intra-renal ROS generation is also potential mechanism contributing to development of acute interstitial nephritis. Experimentally,

ip administration of diclofenac (15 mg/kg) injection for 3 day has been reported to induce renal failure in rats.[152]

7. Osmosis-induced acute renal failure

Osmotic nephrosis is described on the morphological basis and is characterized by vacuolization and swelling of the renal proximal tubular cells. Clinically, osmotic nephrosis is due to intravenous infusion of hypertonic sucrose, hydroxyethyl starch, dextrans, and contrast media to reduce intra-cranial pressure.[153] In preclinical studies, osmotic diuresis is produced by administering 20% sucrose solution in rabbits that produces renal failure within the hour, and is characterized by vacuolar degeneration and nuclear shrinkage of tubular cells.[154] Zhang et al. have reported that single dose administration of mannitol (4%, 9%, 19% and 27%) with dose of 5 ml/kg leads to induction of renal apoptosis and acute renal damage in spontaneous hypertensive rats.[155]

8. Ifosfamide-induced acute renal failure

Ifosfamide (IFO), a synthetic analog of cyclophosphamide, is an alkylating oxazaphosphorine and is widely used as first-line combination therapy for a variety of malignancies including metastatic germ-cell testicular cancer and some sarcomas.[156] High-dose chemotherapy using IFO leads to hemorrhagic cystitis, Fanconi syndrome and ARF. Ifosfamide has been documented to induce the renal failure in patients at higher cumulative doses of 73.5 g/m^2.[157] The other clinical trial report has documented that cumulative ifosfamide dose of 9–128 g/m2/course induces severe renal failure in pediatric osteosarcoma patients.[158] In another study, ifosfamide dose of 12 g/m2 for 6 consecutive days has been shown to develop Nephrotoxicity in 45.46% of patients that required hemodialysis and subsequently 36.36% of patients were reported to die.[159] IFO mustard reacts with deoxyribonucleic acids (DNA) molecules to form intra-and inter-strand cross-links, causing the DNA strand to break and ultimately cell apoptosis and/or necrosis.[160, 161] Ifosfamide has also been shown to inhibit glutathione synthesis, generate reactive oxygen species, mitochondrial damage and apoptosis leading renal failure.[162] Chloroacetaldehyde (CAA, a metabolite of ifosfamide) causes depletion of protein thiol and mitochondrial ATP, DNA cross-links and inhibition of DNA synthesis.[163, 164] Badary has demonstrated that ip administration of ifosfamide at a dose of 50 mg/kg for

5 consecutive days induces the renal damage in rat.[165] ARF has been shown to develop in mice by ip injection with different range of doses such as 350, 550, 800 or 1100 mg/kg of ifosfamide. However, the dose of 550 mg/kg of ifosfamide was reported to produce reproducible ARF within 72 h. Single dose administration of ifosfamide, 550 mg/kg, ip, induced renal failure in more commonly employed animal model to study various aspects of renal dysfunction due to anticancer agents induced renal failure in cancer patients.

9. Uranium-induced acute renal failure

The kidney is being particularly sensitive to uranium. In chronically exposed uranium workers, the reduction in renal proximal tubular reabsorption of amino acids and low molecular weight proteins consistent with uranium nephrotoxicants has been reported.[166] Uranium Nephrotoxicity has been extensively studied in experimental animals using uranyl nitrate (UN) and uranyl acetate, and is characterized by an increased serum creatinine and blood urea nitrogen (BUN) accompanied by abnormal electrolyte excretion, proteinuria, glucosuria and tubular necrosis.[167] As with many nephrotoxins, uranyl-mediated pathologic damage is most evident in the straight position of the proximal tubule.[168] Avasthi et al. reported the development of renal failure in rats by iv administration of two doses of uranyl nitrate 15 mg/kg and 25 mg/kg.[169] Later, researchers have reported the development of renal failure in rats within 5 days by ip administration of single dose of uranyl nitrate (0.5 and 10 mg/kg) dissolved in 0.9% of saline.[170, 171] Fleck et al. reported the development of renal failure in rats by administration of single dose of uranyl nitrate (5 mg/kg, ip) which was dissolved in 0.9% NaCl.[172] It has been reported that single injection of 1 ml/kg (5 mg/kg) into the tail vein of rat induces renal failure.[173, 174] The plasma levels of urea nitrogen and creatinine increase significantly from third day to fifth day after intravenous administration of uranyl nitrate.[175] Choi et al. have reported the development of renal failure in rats within 5 days by iv administration of uranyl nitrate at a dose of 5 mg/kg.[176] Subcutaneous injection of uranyl acetate dihydrate (5 mg/kg) has also been reported to generate ARF within 72 h.[177, 178]

10. Mercuric chloride-induced acute renal failure

Mercuric chloride ($HgCl_2$) is a well-known renal toxicant that causes ARF. A single injection of $HgCl_2$ into rat results in necrosis of the tubular epithelial cells of the

kidney.[179] Early tubular epithelial injury induced by mercuric chloride consists of fragmentation of the plasma membrane, swelling of the mitochondria and disruption of the nucleus and cytoplasmic organelles. Oxidative stress, which occurs after the metabolic generation of ROS, seems to play an important role in the pathogenesis of $HgCl_2$-induced ARF.[180] Zimmermann et al. have reported the development of ARF in rat within 24 h by administration of a single sc injection of $HgCl_2$ at a dose of 2.5 and 4.7 mg/kg.[181] Yoneya et al. have reported the development of ARF in rats within 24 h by ip administration of $HgCl_2$ (1 mg/kg) dissolved in saline (1 mg/ ml).[182] Ewald et al. have also been reporting the development of ARF in mice within 24 h by administration of a single ip injection of $HgCl_2$ at a dose of 6 mg/kg.[183] Ahn et al. has developed renal failure in rabbits within 24 h by administration of a single sc dose of $HgCl_2$ at 10 mg/kg.[184] The development of ARF in rats within 24 h by administration of a single sc dose of $HgCl_2$ at 4.0 mg/kg and 5.0 mg/kg has also been described.[185,186] The administration of mercuric chloride 6 mg/kg, ip (single dose) in rabbit and 10 mg/kg, sc in mice (single dose) induced renal failure models more commonly used to clinically mimic the chemical industrial hazard associated with ARF in human.

11. Potassium dichromate-induced acute renal failure

Chromium is a naturally occurring element found in volcanic dust, rocks, soil, plants and animals. The most common forms of chromium in the environment are hexavalent (Cr6+) and trivalent (Cr3+). Cr6+ and Cr3+ are widely used in industrial and chemical processes such as leather tanning, printing, in hair dyes, steel manufacturing and wood preservative production. In some regions, waste disposal of chromium compounds to the environment contributes to increase its presence and potential toxicity.[187] In biological systems, the soluble forms of Cr6+ are absorbed more easily than Cr3+ and are reduced to Cr3+ via Cr5+ by glutathione, ascorbate and hydrogen peroxide.[188] Once chromium is absorbed, it is distributed in the liver, lung, spleen, kidney and heart. Appel et al. have reported the development of non-oliguric pattern of ARF in rat within 24 h by administration of a single sc injection of potassium dichromate (K2Cr2O7) 15 mg/ kg.[189] Recently, Khan et al. have demonstrated that a single injection of potassium dichromate (15 mg/kg, sc) causes development of ARF within 48 h.[190]

12. Folic acid-induced acute renal failure

Folic acid (FA) induced ARF is a conventional animal model of human ARF.[191] FA-induced renal injury is associated with the rapid appearance of FA crystals within renal tubules and subsequent acute tubular necrosis, followed by epithelial regeneration and renal cortical scarring.[192, 193] The molecular mechanisms by which FA induces ARF remain poorly understood. FA-induced renal failure is characterized by necrosis and apoptosis of tubular epithelial cells. In FA treated animals there is marked reduction in the expression of anti-apoptotic protein B-cell lymphoma-extra large (Bcl-xL) in kidneys along with marked elevation of tumor necrosis factor-a (TNF-a) in blood and kidneys. An iv injection of folic acid (250 mg/kg) is reported to induce ARF after 48 h in mice.[194]

13. Ferric-nitrilotriacetate-induced acute renal failure

Nitrilotriacetic acid (NTA), a synthetic chelating agent, is used as a household and hospital detergent in various countries. NTA is a low-toxic agent;[195, 196] however, the ferric-nitrilotriacetate (Fe-NTA) complex causes acute Nephrotoxicity in animals as well as in humans.[197] Fe-NTA-induced generation of free radicals, including superoxide anions and hydroxyl radicals, is a major mechanism of renal toxicity.[198] Hamazaki et al. have reported that the administration of single dose of Fe-NTA (15 mg iron/kg) induces the acute tubular necrosis and renal failure in rats, while Umemura et al. reported that oral administration of Fe-NTA (12 mg Fe/kg) in rats causes ARF. Furthermore, administration of the single dose of Fe-NTA (8 mg iron/kg, ip) has also been reported to induce renal failure in rats. ARF is induced within 24 h in mice by ip injection of Fe-NTA with different doses such as 1, 2, and 4 mg/kg.[199]

14. S-(1,2-dichlorovinyl)-L-cysteine-induced acute renal failure

S-(1,2-Dichlorovinyl)-L-cysteine (DCVC) is a potent nephrotoxicant and is a metabolite of trichloroethylene (TCE), a ground water contaminant listed as one of the most hazardous chemicals by Agency of Toxic Substances and Disease Registry (ATSDR).[200] DCVC selectively damages the proximal tubules of the kidneys and causes mortality by ARF.[201, 202] Administration of DCVC (30 mg/kg, ip) in mice is reported to cause loss of renal architecture within 24 h.[203] Darnerud et al. have

reported that administration of single dose of DCVC at the lower dose of 5 mg/kg and higher dose of 25 mg/kg produces ARF in mice in a dose dependent manner. Administration of DCVC 5 mg/kg is reported to induce moderate lesions in the straight proximal tubules within 24 h. Furthermore, administration of 25 mg/kg of DCVC is documented to produce more pronounced lesions in the tubular segment that extend to other segments such as sub-capsular region. Wolfgang et al. have reported that two stereoisomers L-DCVC (at 10–5 M) and D-DCVC (at 10–5 M) produce renal injury in vitro system using rabbit renal cortical slices. Furthermore, administration of 25 mg/kg was also reported to produce ARF within 24 and 48 h in rabbits.

15. Sepsis-induced acute renal failure

Cecal ligation and puncture (CLP) induced polymicrobial sepsis is also employed to induce ARF in rats. The rats are anesthetized and a 2 cm ventral midline incision is made to expose and ligate the cecum with a 4.0 silk just distal to the ileocecal valve to avoid intestinal obstruction. Thereafter, ligated cecum is punctured three times with a 16 gauge needle followed by drainage with 3 mm wide latex slice twice and 5 mm width latex slice once. After this procedure, animals are fluid resuscitated with sterile saline (40 ml/kg) and within 24 h the animals develop renal failure as detected by an increase in creatinine levels along with extreme lethargy, diarrhea, piloerection and tachypnea.[204] Ruetten et al. have demonstrated that intravenous infusion of lipopolysaccharide (LPS) (10 mg/kg) for 30 min in the the left femoral vein induces ARF in rat.[205] Johannes et al. have reported that 30 min infusion of LPS (2.5 mg/kg) induces endotoxemia associated renal failure in rat.[206] Jesmin et al. have demonstrated that single ip injection of LPS derived from Escherichia coli (E. coli 055:B5) (15 mg/kg) induces the potential ARF in rats.[207] Recently, the renal artery occlusion along with sc injections of Escherichia coli in 4 week old rats is reported to cause renal failure.[208] An ip administration of bacterial toxic protein, i.e., LPS 15 mg/kg (single dose) induced renal failure in rats is more commonly employed animal model that mimics the infectioninduced renal failure in humans.

16. Bipyridyls-induced acute renal failure

Paraquat and diquat dibromide are commercially available herbicides and are extensively used worldwide. Diquat is useful for studying the effects of ROS in vivo particularly in renal system.[209] It stimulates cellular production of ROS by undergoing cyclic reduction-oxidation processes, in which the diquat dication is reduced to the monocation radical, which inturn reduces molecular oxygen to superoxide. Lock and Ishmael has demonstrated that administration of paraquat (680 µmol/kg, po, and 108µmol/kg, sc) cause the renal tubular damage after 6 and 24 h, respectively, in rats.[210] Diquat (680 µmol/kg, po)-induced renal tubular damage is characterized by urinary proteinuria and glucosuria within 6 to 24 h in rats. It has been reported that a single oral dose of diquat (540µmol/kg) induces the renal functional changes and kidney damage in rats.[211] Rogers et al. have demonstrated that cumulative dose 0–50 µmol/ kg of diquat ip during the period of 6 h induces the ARF in glutathione reductase - deficient mice, with the dose of 7.5 µmol/kg diquat, renal injury is mainly demonstrated in proximal tubules within 1 h and tubular necrosis is observed within 2 hours.

2.5 CISPLATIN[212, 213]

Cisplatin, Cisplatinum, platamin, neoplatin, cismaplat or cisdiamminedichlorid eplatinum (II) (CDDP) is a chemotherapy drug. It was the first member of a class of platinum-containing anti-cancer drugs, which now also includes carboplatin and oxaliplatin. These platinum complexes react in the body, binding to DNA and causing the DNA strands to crosslink, which ultimately triggers cells to die in a programmed way.

Chemical structure

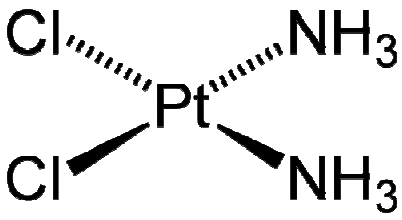


Fig. No: 5

Formula	:	$Cl_2H_6N_2Pt$
Melting point	:	>270 ℃
Density	:	3.738 g/m3
Molar mass	:	300.01 g/mol
IUPAC ID	:	(*SP*-4-2)-diammine dichloro platinum (II)

SYNTHESIS

The synthesis of Cisplatin starts from potassium tetrachloroplatinate. The tetraiodide is formed by reaction with an excess of potassium iodide. Reaction with ammonia forms $K_2[PtI_2(NH_3)_2]$ which is isolated as a yellow compound. When silver nitrate in water is added insoluble silver iodide precipitates and K_2 [Pt $(OH_2)_2$ $(NH_3)_2$] remains in solution. Addition of potassium chloride will form the final product which precipitates. In the tri-iodo intermediate the addition of the second ammonia ligand is governed by the trans effect.

USES

Cisplatin is a widely used and highly effective cancer chemotherapeutic agent. Which is used to treat various type of cancers. One of the limiting side effects of Cisplatin use is nephrotoxicity. Research over the past 10 years has uncovered many of the cellular mechanisms which underlie Cisplatin-induced renal cell death. It has also become apparent that inflammation provoked by injury to renal epithelial cells serves to amplify kidney injury and dysfunction *in vivo*.

2.6 LIPOIC ACID (LA) [214]

It is also known as α-lipoic acid (ALA) and thioctic acid. It is an organosulfur compound derived from octanoic acid. α-lipoic acid (ALA) is made in animals normally, and is essential for aerobic metabolism. It is also manufactured and is available as a dietary supplement in some countries where it is marketed as an antioxidant, and is available as a pharmaceutical drug in other countries.

Chemical structure

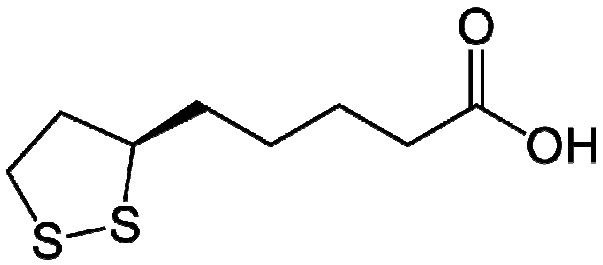


Fig. No: 6

Formula	:	$C_8H_{14}O_2S_2$
IUPAC name	:	(*R*)-5-(1,2-Dithiolan-3-yl) pentanoic acid
Melting point	:	46–48 °C (115–118 °F; 319–321 K)
Molar mass	:	206.33 g/mol

Role of Lipoic acid in Cisplatin induced Nephrotoxicity[215]

Cisplatin treatment elevates Malondialdehyde (MDA) and declined reduced glutathione (GSH). And it will decreases catalase, superoxide dismutase (SOD) and Glutathione peroxidase (GPx) enzymes.

Alpha lipoic acid (ALA) decreases the MDA and increases the GSH, catalase, SOD and GPx levels. Alpha lipoic acid (ALA) increases mRNA expression of catalase, CuZn SOD and GPx genes near to normal compared to Cisplatin-treated mice. Alpha lipoic acid (ALA) may play Nephroprotective role on Cisplatin-induced Nephrotoxicity through antioxidant and anti-apoptotic mechanisms combined with initiation of mRNA expression of antioxidant genes.

2.7 *TAMARINDUS INDICA* LINN

Fig. no: 7 Plant of *Tamarindus indica* Linn with fruits and seeds

2.7.1 Plant monograph[216]

Botanical classification

Kingdom	-	Plantae
Sub kingdom	-	Tracheobionta
Super division	-	Spermatophyta
Division	-	Magnoliopsida
Sub class	-	Rosidae
Super order	-	Rosanae
Order	-	Fabales
Family	-	Fabaceae
Sub family	-	Caesalpinioideae
Genus	-	Tamarindus Linn
Species	-	T.indica
Tribe	-	Detarieae
Binomial name	-	*Tamarindus indica* Linn

Synonyms

Tamarindus occidentails, Tamarindus officinalis hook, Tamarindus umbrosa salisb

Biological source

Whole plant of *Tamarindus indica* Linn

Family

Fabaceae* / *Caesalpinioideae

Vernacular names

Tamil	-	Ambilam, Tindiruni, Puli
Sanskrit	-	Amla, Amli, Amlika, Tintiri
Telugu	-	Chinta, Chintapandu, Sinnta
Hindi	-	Ambli, Imli
Kannada	-	Amla, Amli, Gotu, Hunase, Hannu, Hunise mara
Malayalam	-	Amlam, Madhurappuli, Puli
English	-	Indian date, Sweet tamarind, Tamarind

Parts used

Fruit pulp

Geographical Distribution

Tamarindus indica Linn *(*family - *Fabaceae*) is distributed in Africa, America, Mexico, Asia and Arabian countries.

Morphology

Habitat

- It is a large, slow growing, long living ever green tree with a trunk of diameter up to 1.5 -2.0 meter.
- It can grow 20 – 30 meter height.
- Bark is brown-gray coloured.
- It can tolerate diversity of soils like loam, sandy and clay soil.
- Slightly acidic soil is best for its growth.

Foliage

- Leaves are elliptical ovular, alternate, pinnate with reticulate venation.
- It is a mass of bright green, dense foliage with feathery appearance.

- During hot season the leaf may be shed briefly in dry areas.
- Leaves are 7.5 – 15 cm length, each having 10 to 20 pairs of oblong leaflets (1.25 – 2.5 cm) and 5 – 6 mm wide.
- Leaves fold in cold damp weather and after sunset, due to the degeneration of *lupeol* in dark, which is synthesized in light.

Flowers

- The flowers are inconspicuous, inch-wide, five-petal and are borne in small racemes and are yellow with red streaks.
- Due to the outer colour of the four sepals the flower buds pink, and which are shed when the flower opens.

Fruits

- Fruits are usually between 5 – 14 cm in length and approximately 2 cm wide.
- It is an indehiscent legume, with a hard, brown shell called pods.
- Along with the new branches, there is abundant growth of irregularly curved pods.
- On maturation of pod, the flesh becomes brown or reddish brown and is filled with somewhat juicy, acidulous pulp.
- Fruit ripe fully, the shells are brittle and easily broken.
- The pulp has pleasing sweet or sour flavor along with high content of acid and sugar.
- It is also rich in vitamin B and calcium content.
- The pulp dehydrates to a sticky paste, enclosed by a few course strands of fibres.
- The pods may contain 1 – 12 large flat, glossy brown, obviate seeds embedded in the brown, edible pulp.
- Asian types have longer pods with 6 – 12 seeds, while the African and west Indian types have shorter pods, containing only 3 – 6 seeds.

Phytochemistry

Preliminary phytochemicals study of *Tamarindus indica* have revealed presence of phenolic compounds, cardiac glycosides, mallic acid, tartaric acid,uronic acid, mucilage, pectin, arabinose, xylose, galactose and glucose.

Tamarind plant shows the presence of various essential elements like arsenic, calcium, cadmium, copper, iron, sodium, manganese, magnesium, potassium, phosphorus, lead and zinc.

The ethanolic extract of *Tamarindus indica* showed the presence of fatty acid, out of which 21 are saturated fatty acids such as n-heptadeconate, hexadeconic acid, n-nonadecanoate, etc. along with 11 unsaturated fatty acids such as nenodecenoic acid, 10-octadecenoic acid, heptadeconoate, etc

Fruit pulp contains organic acids such as tartaric acid, acetic acid, citric acid, formic acid, mallic acid and succinic acid. And also the pulp shows the presence of high amounts of ascorbic acid, vitamin B_1, B_3, amino acids such as alanine, phenylalanine, proline, serine, leucine; volatile oils; pectin; proteins and fat.

Pulp also contains alkaloids, glycosides, saponins, sesquiterpenes, flavonoids, tannins and phlobatannins.

Traditional uses

- The raped and un-raped fruits pulp mixed with milk, honey or lemon juice were used as laxative and constipation reliever.
- The leaves and bark were used as wound healer.
- Fresh fruits are used as antipyretic.
- Fruit pulp and leaves are used as anti-malarial agent.
- Flowers, leaves, bark and fruit pulps were used as aphrodisiac.
- Fresh bark or stem used as abdominal pain reliever.
- Fruit pulp with lemon or milk and leaf juice were used as anti diarrheal and anti-dysentry agent.
- Bark and leaves are used as anti asthmatic and anti-tussive.
- Leaves or fruits are used as anti-measles and against mumps.
- Leaf and bark decoctions were used as hepato-protective.
- Leaves are used as anti diabetic.
- Fruits are used as antibacterial.

- Bark is used as anthelminitic.
- Fruits were used as preservative.
- Antiepileptic.
- Antiemetic.
- Antihypertensive.
- Anti-stomachic, and
- Nephroprotective.

2.7.2 Earlier works reported on *Tamarindus indica* Linn

- Bibekananda mehar *et al.,* [217] have done Evaluation of hepatoprotective and invivo antioxidant activity of *Tamarindus indica* Linn (*fabaceae*) seeds extracts in streptozotocin induced diabetic rats. Diabetis induced hepatic damage was evaluated by the serum markers such as SGOT, SGPT, ALP, Bilirubin. Further the effect of HAETIS and AETIS on oxidative stress was determined by the markers of oxidative stresses like Lipid peroxidation (LPO), Determination of super oxide dismutase (SOD), Determination of catalase (CAT), glutathione (GSH), were estimated in liver tissue. HAETIS and AETIS at a dose level of 100mg/kg and 200 mg/kg produce significant ($P< 0.05$) hepatoprotection by decreasing the activity of serum enzymes, bilirubin and lipid peroxidation, while they significantly increase the level of glutathione, super oxide dismutase (SOD) and catalase in a dose dependant manner. Histopathological studies supports the above finding results indicate that hydroalcoholic extracts of *Tamarindus indica* at 200 mg/kg showed more significant hepatic protection as compared to other extracts.

- Narendar Koyagura *et al,* [218] have done Antidiabetic and hepatoprotective activities of *Tamarindus indica* fruit pulp in alloxan induced diabetic rats. The objective of this study was to investigate antidiabetic, hypolipidemic and hepatoprotective activity of ethanolic extract of *Tamarindus indica* fruit pulp in alloxan induced diabetic rats. Animals were made diabetic by injection of single dose of alloxan in three test groups and after that they were treated with ethanolic extract of fruit pulp of *Tamarindus indica* 300 and 500 mg/kg/body weight orally and metformin 150 mg/kg body weight orally respectively for 14 days. Anti-diabetic activity was estimated by measuring serum glucose and lipid profile and hepatoprotective activity was measured by estimating serum liver enzyme levels and histopathological changes in liver tissues. Results were analyzed by One way ANOVA followed by Scheffe multiple comparison tests ($p<0.01$). The two dose levels of *Tamarindus indica* significantly altered alloxan induced changes in serum glucose, lipid profile and serum enzyme levels. But in liver histopathology, higher dose (500 mg/kg) of plant showed complete regeneration whereas lower dose (300 mg/kg) showed only partial improvement in liver histopathology profile.

Present study revealed that *Tamarindus indica* possesses anti-diabetic and hepato protective activity in alloxan induced diabetic rats.

- Abubakar MG *et al.,* [219] have done Acute toxicity and antifungal studies of ethanolic leaves, stem and pulp extract of *Tamarindus indica*. The acute oral toxicity studies of the pulp extract of *Tamarindus indica* at 3000mg/kg and 5000mg/kg body weight of resulted in no mortality. This suggests that the LD_{50} is greater than 5000mg/kg body weight and can be classified as practically non-toxic and considered safe by the recommendations of World Health Organization (WHO) and Organization for Economic and Cultural Development (OECD). Antifungal activity of ethanolic extract of *Tamarindus indica* (leaves, stem bark and pulp) against A. niger, A.flavus and F.oxysporum was studied. The result showed a dose dependent increase in inhibition of growth of these organisms. Of the three plant part the stem bark did not inhibit growth of A. niger and slightly inhibited the growth of A.flavus and F.oxysporum. From this study we can conclude that the pulp and especially the leaves of *Tamarindus indica* could be a promising antifungal agent and the result confirms the use of this plant in traditional medicine for the treatment of fungal infections.

- Prabhu K.H *et al.,* [220] have done Eco-dyeing using *Tamarindus indica* L. seed coat tannin as a natural mordant for textiles with antibacterial activity. *Tamarind* seed coat tannin was extracted and its tannin class was determined. The extracted tannin was employed as a natural mordant alone and in combination with metal mordant namely copper sulphate for cotton, wool and silk fabrics and dyed using natural dyes namely turmeric and pomegranate rind. The colour strength, colour coordinates, wash and light fastness were evaluated and compared for all the three fabrics with and without mordanting. The pre-mordanted fabrics on dyeing gave better colour strength, wash and light fastness than those dyeing obtained without mordanting. The total phenolic content of the extract was calculated and minimum inhibition concentration was 1% against both the Staphylococcus aureus and Escherichia coli bacteria. The mordanted and dyed fabrics resulted in good antibacterial activity up to 20 washes, when natural mordant

was used along with 0.5% and 1% copper sulphate mordant and dyed with natural dyes.

- Doughari J.H *et al.,* [221] have done Antimicrobial Activity of *Tamarindus indica* Linn. To evaluate the scientific basis for the use of the plant, the antimicrobial activities of extracts of the stem bark and leaves were evaluated against some common gram negative and gram positive bacteria and fungi. The study also investigated the chemical constituents of the plant and the effect of temperature and pH on its anti microbial activity. The antimicrobial activity of the concentrated extracts was evaluated by determination of the diameter of zone of inhibition against both gram negative and gram positive bacteria and fungi using the paper disc diffusion method. Results of the phytochemical studies revealed the presence of tannins, saponins, sesquiterpenes, alkaloids and phlobatamins and the extracts were active against both gram positive and gram negative bacteria. The activity of the plant extracts were not affected when treated at different temperature ranges (4oC, 30oC, 60oC and 100oC), but was reduced at alkaline pH. Studies on the minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of the extracts on the test organisms showed that the lowest MIC and the MBC were demonstrated against Salmonella paratyphi, Bacillus subtilis and Salmonella typhi and the highest MIC and MBC was exhibited against Staphylococcus aureus. *Tamarindus indica* has broad spectrum antibacterial activity and a potential source of new classes of antibiotics that could be useful for infectious disease chemotherapy and control.

- Debasis De *et al.,* [222] have done Searching for anti-hyperglycemic phytomolecules through bioassay-guided solvent fractionation and subfractionation from hydro-methanolic (2:3) extract of *Tamarindus indica* Linn seeds in streptozotocin-induced diabetic rat. The study identified the most effective fraction and subfraction of hydro-methanolic extract (2:3) of the seed of *Tamarindus indica* Linn having anti-diabetic activity in rats with diabetes induced by streptozotocin (STZ). The effective fraction and subfraction of *Tamarindus indica* were subjected to an anti-diabetic study in STZ-induced diabetic rats at two dose levels, 100 mg/kg and 25 mg/kg body weight twice a day. Serum insulin, glycosylated hemoglobin, carbohydrate

metabolic enzymes, and transaminases were assessed and the histopathology of the pancreas was examined after 8 weeks of treatment and compared to the vehicle control. Treatment of n-hexane fraction at a dose of 100 mg/kg twice a day for 56 days in STZ-induced diabetic rat resulted in a significant reduction in fasting blood glucose and glycosylated hemoglobin levels along with a rise in serum insulin and glycogen contents in hepatic and skeletal muscle in comparison to chloroform, ethyl acetate, or n-butanol fraction treated groups as well as the untreated diabetic group. The most antidiabetic activity of n-hexane fraction had been highlighted by the activities of hexokinase, glucose-6-phosphate dehydrogenase, glucose-6-phosphatase, and lactate dehydrogenase in the liver, kidney, cardiac, and skeletal muscle in respect with groups treated with other fractions. Two subfractions, A and B, were obtained from the n-hexane fraction using petroleum ether, of which subfraction B was more bioactive considering the above biosensors and was comparable with glibenclamide, a standard antihyperglycemic drug. Chromatographic study by high performance thin layer chromatography focused on two components of subfraction B, P1 and P2 where P1 is predominant, conformed by high performance liquid chromatography. The dose of subfraction B was 25 mg/kg twice a day i.e., 1/4 dose of the n-hexane fraction. The n-hexane fraction and subfraction B of *Tamarindus indica* are free from hepatic and renotoxicity according to the study of serum transaminase.

- Iain E.P *et al.,* [223] have evaluated X-ray diffraction studies on the xyloglucan from tamarind (*Tamarindus indica*) seed. Oriented samples of the xyloglucan polysaccharlde from tamarind seed were examined using X-ray diffraction. Periodicities indexing on a spacing of 2.06 nm were observed along the chain direction. This value is twice that reported for cellulose and is commensurate with four j-1, clinked glucose residues. Flat, ribbon like, two fold helical models for the b- 1, Clinked polyglucose backbone, with two possible schemes for decoration with xylose and galactose side groups, are proposed.

- Samina kabir khanzada *et al.,* [224] have studied chemical constituents of *tamarindus indica* medicinal plant in sindh. Thirty two fatty acids, two other compounds 9ß, 19-Cyclo-4 ß4, 4, 14, χ-trimethyl-5ά-cholestan3ß-ol, 24R-Ethyl cholest-5-en, 3β-ol and 12 essential elements viz., Arsenic, Calcium, Cadmium, Copper, Iron, Sodium, Manganese, Magnesium, Potassium, Phosphorus, Lead, and Zinc were isolated from *Tamarindus indica* medicinal Plant. Accumulation of Copper was the lowest in *Tamarindus indica* while Potassium present with highest accumulation. Total protein in *Tamarindus indica* was 7.5 to 6.6 %.

- Oranuch Nakchat *et al.,* [225] have done Tamarind seed coat extract restores reactive oxygen species through attenuation of glutathione level and antioxidant enzyme expression in human skin fibroblasts in response to oxidative stress. To investigate the role and mechanism of tamarind seed coat extract (TSCE) on normal human skin fibroblast CCD-1064Sk cells under normal and oxidative stress conditions induced by hydrogen peroxide (H_2O_2). Tamarind seed coats were extracted with boiling water and then partitioned with ethyl acetate before the cell analysis. Effect of TSCE on intracellular reactive oxygen species (ROS), glutathione (GSH) level, antioxidant enzymes such as superoxide dismutase (SOD), glutathione peroxidase (GPx) and catalase activity including antioxidant protein expression was investigated. TSCE significantly attenuated intracellular ROS in the absence and presence of H_2O_2 by increasing GSH level. In the absence of H_2O_2, TSCE significantly enhanced SOD and catalase activity but did not affected on GPx. Meanwhile, TSCE significantly increased the protein expression of SOD and GPx in H_2O_2-treated cells. Conclusions: TSCE exhibited antioxidant activities by scavenging ROS, attenuating GSH level that could protect human skin fibroblast cells from oxidative stress. Our results highlight the antioxidant mechanism of tamarind seed coat through an antioxidant enzyme system.

- Tahiry Ranaivoson *et al.,* [226] have done Distribution, biomass and local importance of tamarind trees in south-western Madagascar. The multipurpose tamarind (*Tamarindus indica* L.) tree is important for people's livelihood and considered as sacred in the Mahafaly region of south-western Madagascar. However, the ongoing over exploitation of this species has caused a decline

of tamarind trees. In this study, the species distribution, changes in tamarind biomass and the role of traditional taboos for the conservation of this species were determined to identify opportunities and constraints for its conservation and appropriate land management planning. Semi structured interviews (N =63) were conducted in 10 villages in the study region to obtain information regarding the utilization of tamarind trees. During field surveys, the diameter at breast height (DBH), height, wood volume and wood biomass were measured for already felled trees (N = 25). Additionally, 318 trees were inventoried by measuring their DBH, height and GPS location. Using high resolution satellite images from 2004/2005 and 2012 the crown areas of all tamarind trees in six village areas were identified. Allometric equations were established to predict their wood biomass from DBH, crown surface and wood volume. Tamarind trees are mainly used as supplementary food, as well as for traditional ceremonies, charcoal production and medicinal purposes. Altogether, 0.06–0.35 trees ha^{-1} were observed. A regression analysis yielded high coefficients of determination for the relationships between DBH and wood biomass (r2 = 0.98), DBH and crown area (r2 = 0.72), and crown area and wood biomass (r2 = 0.71). From 2004/2005 to 2012, wood biomass losses of 12%–90% were caused by charcoal production and slash and burn agriculture. The traditionally sacred status of the tree has become insufficient to secure its conservation in the Mahafaly region.

- Pinar Kuru *et al.,* [227] have done *Tamarindus indica* and its health related effects. *Tamarindus indica* belongs to the family *Fabaceae*, commonly known as Tamarind tree, is one of the fruit tree species that is used as traditional medicine. The aim of this article is to review the current literatue on health related effect of *Tamarindus indica*. Literature review about this plant was conducted between 2003 and 2014 through Pubmed and Google. Only the health related articles selected. Tamarind tree is found especially in the Indian subcontinent, Africa, Pakistan, Bangladesh, Nigeria and most of the tropical countries. It is preferred to be used for abdominal pain, diarrhea and dysentery, some bacterial infections and parasitic infestations, wound healing, constipation and inflammation. It is a rich source of most of the essential amino acids and phytochemicals, and hence the plant is reported to possess

anti-diabetic, antimicrobial, anti-venomic, antioxidant, antimalarial, cardio protective, hepatoprotective, antiasthmatic, laxative and anti-hyper lipidemic activity. *Tamarindus indica* has ameliorative effects on many diseases. It can also be preferred as a nutritious support for malnourished.

3. SCOPE OF THE PRESENT STUDY

Kidneys play an important part in the maintenance of our endocrine and acid-base balance, blood pressure, erythropoiesis etc. The main functions of kidney can be categorised as formation of urine, water and electrolyte balance and production of hormones and enzymes. Kidneys have some delicate tasks, especially when they have to deal with unwanted substances, which they have to clear from the system.

Nephrotoxicity can be defined as renal dysfunction that arises as a direct result of exposure to external agents such as drugs and environmental chemicals. It has been known from many years that toxic metals and heavy metals have toxic effects on kidney by accumulating and producing broad spectrum of morphological and functional effects of kidney. A number of drugs like cisplatin and antibiotics including penicillins, cephalosporins, tetracycline, sulfonamides and amino glycosides are known to be potential nephrotoxins.

Several plants have reported which have nephroprotective activity. Selection of scientific and systematic approach to the biological evaluation of plant products based on their use in the traditional systems of medicine and the nature of active principles form the basis for an ideal approach in the development of new drugs from plants. *Tamarindus indicia* Linn plant is scientifically proved for its traditional usage as nephroprotective.

In Ayurveda plants namely milk thistle, drum stick, ginger, tamarind are used as nephroprotective agent. The common active constituents present in these plants, responsible for nephroprotective activity is found to be flavonoids, iso flavonoids, phenolic compounds like tannins etc. The active constituents present in *Tamarindus indica* are flavonoids, phenolic compounds, cardiac glycosides, malic acid, tartaric acid, mucilage, pectin, arabinose, xylose, galactose, glucose and uronic acid. Also the antioxidants present in the plant interact with free radicals and may prevent the damage caused by them.

Literature survey has revealed that the plant of *Tamarindus indica Linn* is prescribed as a nephroprotective, an anti-emitic, an antihistaminic, an antimicrobial, an antifungal, an anti-melioidosis, a defluoridation activity, an analgesic, an anti

pyretic, an anti-inflammatory, an anti-viral, an anti-nematodal, a molluscicidal activity, an anti-diabetic and hypolipidemic, an anti-oxidant activity, a cytotoxic, an antivenom, a hepatoprotective, an anthelmintic, a laxative, but no scientific study has been reported regarding its nephroprotective activity on its fruit pulp. Hence the study was undertaken to investigate the nephroprotective activity of ethanolic extract of the fruit pulp of *Tamarindus indica* Linn.

There is a growing interest of public in traditional medicine, particularly in the treatment of nephrotoxicity partly because of limited choice in the pharmacotherapy. Certain Indian Medicinal plants have been reported to exhibit protective effect of renal tissues against injuries. Since there are only few researches made on this field of nephroprotection, this present study of nephroprotective activity of *Tamarindus indica* will satisfy the research for better and cost effective nephroprotection.

4. AIM AND OBJECTIVES

AIM

The present study was focused on the investigation of Nephroprotective activity of the ethanolic extract of fruit pulp of *Tamarindus indica* Linn on cisplatin induced nephrotoxicity in Wistar albino rats.

OBJECTIVES

The objectives of the present study were,

1. Evaluation of Nephroprotective effect of EETI.
2. Comparison of Nephroprotective effect of standard drug with toxic substance.
3. Comparison of Nephroprotective effect of EETI with standard drug as well as toxic substance.
4. Histopathological studies of all groups of animals to compare the histological damages.

5. PLAN OF WORK

The plan of work for the study of *Tamarindus indica* Linn was carried out as follows:

1. Literature review, identification, authentification and collection of *Tamarindus indica* Linn
2. Preliminary phytochemical study
    A) Preparation of extracts
    B) Qualitative phytochemical studies
3. Pharmacological studies
    - Nephroprotective study of ethanolic extract of fruit pulp of *Tamarindus indica* Linn.
    - Estimations of
        a. General parameters
            - Urine volume
            - Body weight
        b. Serum biochemical parameters
            - Serum Creatinine level
            - Blood urea nitrogen (BUN)
        c. Urine biochemical parameters
            - Creatinine clearence
        d. Oxidative stress parameters
            - Malondialdehyde (MDA)
        e. Enzymatic antioxidant parameter
            - Superoxide dismutase (SOD)
            - Catalase (CAT)
            - Glutathione peroxidase (GPx)
        f. Non enzymatic antioxidant parameter
            - Reduced glutathione (GSH)
4. Histopathology of kidney
5. Statistical analysis and documentation

6. MATERIALS AND METHODS

6.1 SELECTION OF THE PLANT

The medicinal plant *Tamarindus indica* Linn (Family: *Fabaceae*) was selected for Nephroprotective activity based on the literature survey.

6.1.1 Collection and authentication of the plant

The fruit pulp of *Tamarindus indica* Linn was collected from Valanchery, Kerala. The plant *Tamarindus indica* Linn belonging to the family of *Fabaceae* was identified and authenticated by Prof. Dr. A. Balasubramanian, Ph.D., Director of A.B.S botanical conservation, Research and Training centre, kaaripatti, Salem district, Tamilnadu-636 106.

6.1.2 Shade drying and cutting of the fruit pulp

The fruit pulp of *Tamarindus indica* Linn were collected and shade dried at the room temperature and then cut it in to small pieces, which was used for the extraction for further studies.

6.2 MACERATION

Fresh fruit pulps of *Tamarindus indica* Linn were cut into small pieces, seeds were removed and air dried. The dried pieces of *Tamarindus indica* Linn fruit pulp, weighing 100 g, were soaked in 500 ml of 95% ethanol in a round bottom flask for about 24 hours.

6.3 EXTRACTION

Extracting values of crude drug are useful for their evaluation, especially when the constituents of a drug cannot be readily estimated by any other means. Further, these values indicate the nature of the constituents present in a crude drug

Ethanolic extract

Solvent - Ethanol

The process of extraction was done by reflux condensation method using soxhlet apparatus at 60-80 ºC for 9 hours. The extract was concentrated by distillation apparatus till a syrupy consistency was obtained. Finally, the extract was put in a china dish and evaporated at40-60 ºC temperature in a water bath, 22 gms of semisolid extract was obtained.[228]

Table-2

Nature and colour of Ethanolic extract of *Tamarindus indica* Linn

S. No	Name of the plant extract	Part used
1.	*Tamarindus indica* Linn	Fruit pulp

6.3.1 Preliminary phytochemical screening [229, 230]

The Ethanolic extract of the fruit pulp of *Tamarindus indica* Linn was subjected to a preliminary phytochemical screening to identify the active chemical constituents.

Test solution

The Ethanolic extract of the fruit pulp of *Tamarindus indica* Linn was taken and dissolved in distilled water.

6.3.1.1 TEST FOR CARBOHYDRATE

A small quantity of the extract was dissolved in 4 ml of distilled water and filtered. The filtrate was subjected to the following tests to detect the presence of carbohydrate and glycosides.

Molisch's test

Few drops of Molisch's reagent were added to an aqueous solution of each extract followed by vigorous shaking. Thereafter, 1.0 ml of conc. H_2SO_4 was added carefully by sliding down the walls of the tube gently to form two layers. There is an appearance of brown ring separating the solution into two layers, it indicate the presence of carbohydrate.

Fehling's test

To 1.0 ml of aqueous solution of each extract was added 3.0 ml of a mixture of equal volumes of Fehling's solutions A and B and boiled in a water bath at about 40 ℃ for 2 min. A brick red colour at the bottom of the test tube was an indication of the presence of reducing sugar.

Anthrone test

2 mg of the extracts was shaken with 10ml water, filtered concentrated. To this 2ml of anthrone reagent solution was added. Formation of green or blue colour indicated presence of carbohydrates.

6.2.1.2 TEST FOR GLYCOSIDES

Tests for glycosides were performed as follows:

(i) To 0.1 g of each extract in a test tube was added 5.0 ml of water and the mixture heated in a water bath at 100 ℃ for 2 min. The mixture was filtered through a Whatman No. 1 filter paper. A mixture of Fehling's solutions A and B were added to the filtrate until it became alkaline: followed by heating for 2 min.

(ii) The above procedure was repeated, except that 5.0 ml of dilute sulphuric acid was added to 0.1g of the extract instead of water: and the quantity of precipitate formed was noted.

(iii) About 0.1 g of each extract was put into a stoppered conical flash in which was suspended a strip of sodium picrate paper. The flask was warmed gently for about an hour at 37 ℃ and allowed to stand. The test paper was examined for any change in colour.

Legal's and Borntrager's tests

Another portion of the extract was hydrolysed with hydrochloric acid for a few hours on a water bath and then hydrolysate was subjected to Legal's and Borntrager's tests to detect the presence of different glycosides.

Legal' test

To the hydrolysate, 1ml of pyridine and a few drops of sodium nitroprussside solutions were added and then it was made alkaline with sodium hydroxide solution. Appearence of pink to red colour showed the presence of glycosides.

Borntrager's test

Hydrolysate was treated with chloroform and then the chloroform layer was separated. To this equal of the diluted ammonia solution was added. Ammonia layer acquired pink colour, showing the presence of glycosides.

6.2.1.3 TEST FOR TANNINS

Small quantity of the extract was taken in water and test for the presence of tannins was carried out with the following reagents.

Ferric chloride test

A 5%solution of ferric chloride in 90% of alcohol was prepared. Few drops of this solution was added in to a little of the above filtrate. Dark green or deep blue colour is obtained, it indicate the presence of tannins.

Lead acetate test

A 10% w/v solution of basic lead acetate in distilled water was added to the test filtrate. Precipitate is obtained, presence of tannins.

Potassium dichromate test

If on an addition of solution of potassium dichromate in test filtrate, dark colour is developed, tannins are present.

6.2.1.4 TEST FOR SAPONINS

Presence of saponins was determined by their frothing property as well as capacity to form emulsion with oils. (i) For the frothing test, about 5 mg of extract was shaken vigorously with sodium bicarbonate and examined for frothing; (ii) For the emulsification test, 2 drops of olive oil was added to 5.0 mL of aqueous solution of

the extract in a test, shaken vigorously and observed for formation of an emulsion. The control was without extract but water and olive oil.

Foam test

The extract was diluted with 20 ml of distilled water and it was agitated in a graduated cylinder for 15minutes. There is formation of one layer of foam, which showed the presence of saponins.

6.2.1.5 TEST FOR FLAVANOIDS

With aqueous sodium hydroxide solution

Small quantity of the extract was dissolved in aqueous sodium hydroxide. Yellow colour was produced which indicated the presence of flavones.

With conc. sulphuric acid

To the small portion of extract, concentrated sulphuric acid was added. Yellow to orange colour was produced which indicated the presence of flavones.

Shinoda's test

Small quantity of the extract was dissolved in ethanol. To them pieces of magnesium were added followed by conc. hydrochloric acid drop wise added and heated. Appearance of magenta colour showed the presence of flavonoids.

6.2.1.6 TEST FOR ANTHROQUINONES

Approximately 0.1 g of the extract was mixed with 5.0 ml of chloroform and agitated for 5.0 min. The solution was filtered and equal volume of ammonia was added to the filtrate and agitated again. A brick red colour in the upper aqueous layer indicates the presence of free anthroquinones.

6.2.1.7 TEST FOR ALKALOIDS

Dragendroff's test

To the extract Dragendroff's reagent (potassium bismuth iodine solution) was added. A reddish brown precipitate was produced, which indicated the presence of alkaloids.

Mayer's test

To the extract Mayer's reagent (potassium mercuric iodine solution) was added. A reddish brown precipitate was produced, which indicated the presence of alkaloids.

Wagner's test

To the extract Wagner's reagent (iodine- potassium iodide solution) was added. A reddish brown precipitate was produced, which indicated the presence of alkaloids.

Hagner's test

To the extract Hagner's reagent (Saturated solution of picric acid) was added. A yellow precipitate was produced, which indicated the presence of alkaloids.

Tannic acid test

To the extract tannic acid solution was added. A buff colour precipitate was produced, which indicated the presence of alkaloids.

6.2.1.8 TEST FOR PHYTOSTEROL AND TERPENES

A 1.0 g weight of the extract was mixed with 5.0 ml of 95% ethanol and then filtered. The filtrate was evaporated to dryness and the residue re-dissolved in 5.0 ml of anhydrous chloroform and then filtered. The latter filtrate was divided into two portions for the following tests:

Liebermann-Burchard Test

The first portion was mixed with 1 ml of acetic anhydride followed by the addition of 1.0 ml of concentrated Sulfuric acid gently down the side of the test tube to form a layer underneath. The formation of a reddish violet colour at the junction of the two liquids and a green colour in the chloroform layer would indicate the presence of terpenes.

Salowski's Test

To the second portion of the solution was added 2.0 ml of concentrated Sulfuric acid carefully down the side of the tube so that the sulfuric acid formed a layer. No reddish brown colour at the interface would indicate the absence of sterols.

The results of phytochemical screening were shown in Table no.5

6.3 Pharmacological Evaluation

6.3.1 ACUTE TOXICITY STUDY[231]

Whenever an investigator administers a chemical substance to a biological system, different types of interactions can occur and a series of dose-related responses result. In most cases these responses are desired and useful, but there are a number of other effects which are not advantageous. These may or may not be harmful to the patients. The types of toxicity tests which are routinely performed by pharmaceutical manufacturers in the investigation of new drug involve acute, sub-acute and chronic toxicity. Acute toxicity is involved in estimation of LD50 (the dose has proved to be lethal (causing death) to 50% of the tested group of animals).

Determination of oral toxicity is usually an initial screening step in the assessment and the evaluation of the toxic characteristics of all compounds. This article reviews the methods of so far utilized for the determination of median lethal dose (LD50) and the new changes which would be made. This has to go through the entire process of validation with different categories of substances before its final acceptance by regulatory bodies.

Organisation for Economic co-operation and Development (OECD) regulates guidelines for oral acute toxicity study. It is an international organisation which works with the aim of reducing both the number of animals and the level of pain associated with acute toxicity testing. To determine the acute oral toxicity OECD frames the following guideline methods.

OECD 401 – Acute Oral Toxicity

OECD 420 – Acute Oral Toxicity: Fixed Dose procedure

OECD 423 –Acute Oral Toxicity: Acute Toxic Classic method

OECD 425 – Acute Oral Toxicity: Up and own Procedure

In the present study the acute oral toxicity of *Spermacoce ocymoides* Burn f was carried out according to OECD 423 guideline (Acute Oral Toxicity: acute Toxic Classic Method).

ACUTE ORAL TOXICITY

Acute oral toxicity refers to those adverse effects that occur following oral administration of a single dose of a substance or multiple doses given within 24 hours.

LD_{50} (median lethal oral dose)

LD_{50} (median lethal oral dose) is a statistically derived single dose of a substance that can be expected to cause death in 50 per cent of animals when administered by the oral route. The LD_{50} value is expressed in terms of weight of test substance per unit weight of test animal (mg/kg).

PRINCIPLE

It is based on a stepwise procedure with the use of a minimum number of animals per step; sufficient information is obtained on the acute toxicity of the test substance to enable its classification. The substance is administered orally to a group of experimental animals at one of the defined doses. The substance is tested using a step wise procedure, each step using three animals of a single sex (normally females). Absence or presence of compound- related mortality of the animals dosed at one step will determine the next step, i.e.;

- No further testing is needed
- Dosing of three additional animals, with the same dose
- Dosing of three additional animals at the next higher or the next lower dose level.

SELECTION OF ANIMAL SPECIES

The preferred rodent species was the rat. Normally females were used. Females were generally slightly more sensitive. Healthy young adult animals of commonly used laboratory strains were employed. Females were nulliparous and non-pregnant. Each animal, at the commencement of it's dosing, were between 8 to 12 weeks old.

ADMINISTRATION OF DOSES

The test substance was administered in a single dose by gavages using a oral feeding needle. Animals were fasted prior to dosing (e.g. with the rat, food but not water should be withheld over-night, with the mouse, food but not water was withheld for 3-4 hours). Following the period of fasting, the animals were weighed and the test substance administered. After the substance has been administered, food was withheld for a further 3-4 hours in rats.

OBSERVATION

Animals were observed individually after dosing at least once during the first 30 minutes, periodically during the first 24 hours, with special attention given during the first 4 hours, and daily thereafter, for a total of 14 days, except where they need to be removed from the study and humanely killed for animal welfare reasons or are found dead. However, the duration of observation was not fixed rigidly. It was determined by the toxic reactions, time of onset and length of recovery period and extended when considered necessary. The times at which signs of toxicity appeared and disappeared were important, when toxic signs were to be delayed. All observations were systematically recorded with individual records being maintained for each animal.

Fixation of doses of the extract

An acute oral toxicity study was carried out according to OECD guidelines. No adverse effect was reported or mortality in albino wister rats up to 2000mg/kg p.o. of ethanolic extracts of *Tamarindus indica* Linn.

Therefore, the maximum tolerated dose 200mg/kg & 400mg/kg was chosen for further studies.

6.3.2 Animals

Albino wistar rats of either sex (150-200 gm) were procured from the animal house of King's institute, Guindy, Chennai, Tamilnadu, India. Prior to the experiment the rats were housed in a clean polypropylene cage (6 rats/cages) for a period of 7 days under temperature (25-30^{0}c), relative humidity (45-55%).

The Institutional Animal Ethics Committee approved the experimental protocol and the conditions in the animal house approved by Committee for Supervision on Experiments on Animals. The study was conducted in accordance with IAEC guidelines (**IAEC approval No: IAEC/XLVIII/03/CLBMCP/2016 dated on 04/05/2016**).

6.3.3 Drugs and chemicals

All the drugs, chemicals and reagents were procured from S.D Fine chemicals, Mumbai, India. All chemicals and reagents used were of analytical reagent.

6.3.4 Experimental protocol[232]

The Nephroprotective activity was tested on five groups of albino wistar rats of either sex, each group consisting of six animals.

Group-I : Served as normal control received 0.5 % DMSO (Dimethyl sulphoxide) ; for 15 days.

Group-II : Served as Nephrotoxic control, received vehicle (0.5% DMSO); for 15 days.

Group-III : Received the standard Nephroprotective drug, (Lipoic acid (50mg/kg; p.o)) dissolved in DMSO for 15 days.

Group-IV : Received ethanolic extract of *Tamarindus indica* Linn (200mg/kg; p.o) dissolved in DMSO for 15 days.

Group-V : Received ethanolic extract of *Tamarindus indica* Linn (400mg/kg; p.o) dissolved in DMSO for 15 days.

On the 10th day 2 hours after the administration of standard Nephroprotective drug (Lipoic acid) and *Tamarindus indica* (200 & 400 mg/kg) II-V groups received cisplatin (7.5mg/kg; i.p).

6.3.5 Blood collection techniques used in the present study[232]

At the end of the experimental period, *ie* on the 15th day animals were sacrificed under mild ether anesthesia. The blood was collected by retro-orbital vein puncture using a fine capillary to an anticoagulant tube and allowed to stand for 30 min at 37℃ and then centrifuged to separate the serum to evaluate the biochemical markers.

6.3.6 Preparation of kidney homogenate[232]

The kidney was quickly removed and perfused immediately with ice-cold saline (0.9% NaCl). A portion of the kidney was homogenized in chilled Tris-HCl buffer (0.025 M, pH 7.4) using a homogenizer. The homogenate obtained was centrifuged at 5000 rpm for 10 minutes, supernatant was collected and used for various biochemical assays.

6.4 ANALYIS OF GENERAL PARAMETERS

6.4.1 Estimation of urine volume

The animals are kept in separate metabolic cages for 24 hours. Each rat urine volume are taken after 24 hours. The food wastes and fecel matters are removed from the urine. And the volume of urine is measured by using measuring cylinder.

6.4.2 Estimation of Body weight

At the end of the experiment, each group of the animals were kept individually in the cages. Remove the food and water, and each animal are individually weighed and the weight were recorded.

6.5 ANALYIS OF SERUM BIOCHEMICAL PARAMETERS

6.5.1 Estimation of Serum Creatinine[233]

Five test tubes were labelled as A, B, C, D and E. Where A&B is taken as standard, C &D taken as test and the E where taken as blank. Into E (blank), 2 ml of distilled water, into C&D (test), 0.5 ml serum and 1.5 ml of water, into A&B (standard), 1.5 ml of water and 0.5 ml of creatinine standard (3mg/dl) were pipette out. 6 ml of picric acid and 0.4 ml of sodium hydroxide (NaOH) (2.5M) were added in all the five test tubes.

Reagents

1. Creatinine stock standard: 150 mg creatinine in 100 ml water (1.5 mg/ml)
2. Creatinine working standard for serum (3mg/dl): dilute 10 ml of stock and make the volume up to 500 ml with water.
3. Serum samples.
4. NaOH (2.5 M).
5. Picric acid.

Table-3

	Standard (A,B)	Test (C,D)	Blank (E)
Serum	-	0.5 ml	-
Distilled water	1.5 ml	1.5ml	2 ml
Standard for serum	0.5 ml	-	-
Picric acid	6 ml	6 ml	6 ml
NaOH (2.5 M)	0.4 ml	0.4 ml	0.4 ml

1. Mix well
2. Add 0.4 ml of 2.5 M NaOH
3. Allow to stand for 20 minutes
4. Read the absorbance against the blank at 520 nm

$$\text{Serum creatinine} = \frac{\text{Absorbance of test}}{\text{Absorbance of std}} \times \text{Concentration of std}$$

6.5.2 Estimation of Serum Blood urea nitrogen (BUN) [234]

The blood urea was estimated by Berthelot method (Fawcett and Scott, 1960) using the commercially available kit (Kamineni Life Sciences Pvt. Ltd. Hyderabad, India). 1000 µl of working reagent-I containing urease reagent, and a mixture of salicylate, hypochlorite and nitroprusside was added to 10 µl of serum, 10 µl of standard urea (40 mg/dl) and 10 µl of purified water to prepare test, standard and blank, respectively. All the test tubes were mixed well and incubated at 37 ℃ for 5 min. Then 1000 µl of reagent-II containing alkaline buffer, was added to all the test tubes, which were incubated at 37 ℃ for 5 min. Urease catalyses the conversion of urea to ammonia and carbon dioxide. The ammonia thus released reacts with a mixture of salicylate, hypochlorite and nitroprusside to yield indo phenol, a blue-green coloured compound. The intensity of the colour produced is directly proportional to the concentration of urea in the sample and is measured spectrophotometrically at 578 nm. The blood urea was calculated using the following formula:

$$\text{Blood urea } \left(\frac{mg}{dl}\right) = \frac{\text{Absorbance of test}}{\text{Absorbance of std}} \times 40$$

$$\text{Blood urea nitrogen } \left(\frac{mg}{dl}\right) = \text{Serum urea } \times 0.469$$

6.6 ANALYIS OF URINE BIOCHEMICAL PARAMETERS

6.6.1 Estimation of Creatinine clearance[235]

Five test tubes were labelled as A, B, C, D and E. Where A&B is taken as standard, C&D taken as test and the E where taken as blank. Into E (blank), 2 ml of distilled water, into C&D (test), 0.5 ml urine and 1.5 ml of water, into A&B (standard), 1.5 ml of water and 0.5 ml of creatinine standard (3mg/dl) were pipette out. 6 ml of picric acid and 0.4 ml of sodium hydroxide (NaOH) (2.5M) were added in all the five test tubes.

1. Creatinine stock standard: 150 mg creatinine in 100 ml water (1.5 mg/ml)
2. Creatinine working standard for urine (0.75 mg/dl): dilute 50 ml of stock and bring the volume up to 200 ml with water.
3. Urine samples.
4. NaOH (2.5 M).
5. Picric acid.

Table-4

	Standard A,B	Test C,D	Blank E
Urine	-	0.5 ml	-
Distilled water	1.5 ml	1.5ml	2 ml
Standard for serum	0.5 ml	-	-
Picric acid	6 ml	6 ml	6 ml
NaOH (2.5 M)	0.4 ml	0.4 ml	0.4 ml

1. mix well
2. Add 0.4 ml of 2.5 M NaOH
3. Allow to stand for 20 minutes
4. Read the absorbance against the blank at 520 nm

$$\text{Creatinine clearance} = \frac{\text{Urinary creatinine}}{\text{Serum creatinine}} \times \text{volume of urine ml/min/1.73 m}^2$$

$$\text{Urinary creatinine} = \frac{\text{Absorbance of test}}{\text{Absorbance of std}} \times \text{Concentration of std}$$

6.7 ANALYIS OF OXIDATIVE STRESS PARAMETERS

6.7.1 Estimation of malondialdehyde (MDA)[236]

Lipid peroxidation (LPO) was assayed by the method of in which the malondialdehyde (MDA) released served as the index of LPO. The extent of LPO in the hepatic tissue was assayed by measuring one of the end products of this process, the thiobarbituric acid-reactive substances (TBARS). As 99% TBARS is malondialdehyde (MDA), thus this assay is based on the reaction of 1 molecule of MDA with 2 molecules of TBARS at low pH (2- 3) and at a temperature of 95℃ for 60 min. The resultant pink chromogen can be detected spectrophonometrically at 532 nm.

Reagents

- ✓ Standard: 1, 1, 3, 3-tetra ethoxypropane (TEP).
- ✓ 8.1% Sodium dodecyl sulphate (SDS)
- ✓ 20%Acetic acid
- ✓ 0.8%Thiobarbituric acid (TBA)
- ✓ 15:1 v/v n-butanol: pyridine mixture

Procedure

To 0.2 ml of tissue homogenate, 0.2 ml of 8.1% SDS, 1.5 ml of 20% acetic acid (pH 3.5) and 1.5 ml of 0.8% TBA were added. The mixture was made up to 4 ml with water and then heated in a water bath at 95.8ºC for 60 min using glass ball as a condenser. After cooling, 1 ml of water and 5 ml of n-butanol: pyridine (15:1 v/v) mixture were added and shaken vigorously. After centrifugation at 4000 rpm for 10 min, the organic layer was taken and its absorbance was measured at 532 nm. The level of MDA was expressed as nmoles of mg of tissue.

6.8 ANALYSIS OF ENZYMATIC ANTIOXIDANTS PARAMETERS

6.8.1 Estimation of superoxide dismutase (SOD) [237]

This enzyme catalyzes the dismutation of superoxide anion ($O2^{-\cdot}$) to hydrogen peroxide and molecular oxygen in the following manner

$$H_2O + 2O_2^{-\cdot} + 2H^+ \rightarrow 2H_2O_2 + O_2$$

The enzyme activity was assayed by the method of Misra and Fridovich, 1972

Reagents

- ✓ 0.1 M Carbonate-bicarbonate buffer; pH 10.2.
- ✓ 0.6 mM EDTA solution
- ✓ 1.8 mM Epinephrine (prepared in situ)
- ✓ Absolute ethanol.
- ✓ Chloroform

Procedure

0.1 ml of tissue homogenate was added to the tubes containing 0.75 ml ethanol and 0.15 ml chloroform (chilled in ice) and centrifuged. To 0.5 ml of supernatant, added 0.5 ml of 0.6 mM EDTA solution and 1 ml of 0.1 M carbonate-bicarbonate (pH 10.2) buffer. The reaction was initiated by the addition of 0.5 ml of 1.8 mM epinephrine (freshly prepared) and the increase in absorbance at 480 nm was measured. The unit for superoxide dismutase (SOD) is nmoles/mg of protein.

6.8.2 Estimation of Catalase (CAT).[238]

This enzyme catalyzes conversion of hydrogen peroxide into water and molecular oxygen.

$$2H_2O_2 \rightarrow 2H_2O + O_2$$

The enzyme activity was assayed by the method of Sinha ,1972.

Reagents

- ✓ Dichromate-acetic acid reagent: 5% potassium dichromate in water was mixed with glacial acetic acid in the ratio of 1:3 (v/v).
- ✓ 0.01 M Phosphate buffer; pH 7.0.
- ✓ 0.2M Hydrogen peroxide

Procedure

0.1 ml of the tissue homogenate was added to the reaction mixture containing 1ml of 0.01 M phosphate buffer (pH 7.0) pre-warmed to 37℃, 0.4 ml of distilled water and the mixture was incubated at 37℃. The reaction was initiated by the addition of 0.5 ml of 0.2 M hydrogen peroxide and the reaction mixture was incubated at 37℃ for one minute. The reaction was terminated by the addition of 2 ml of dichromate-acetic acid reagent after15, 30, 45, and 60 seconds. Standard hydrogen peroxide in the range of 4-20 µl/ moles were taken and treated in the same manner. All the tubes were heated in a boiling water bath for 10 minutes, cooled and the green colour that developed was read at 590 nm against blank containing all components except the enzyme. Catalase activity was expressed in U/mg protein.

6.8.3 Estimation of glutathione peroxidase (GPx) [239]

Glutathione peroxidase activity was determined according to the method of Hafemann *et al.* (1974). The activity of GPx was determined by measuring the decrease in GSH Content after incubating the sample in the presence of H_2O_2 and NaN_3.

$$H_2O_2 + 2\ GSH\ 2H_2O + 2\ GSSG$$

Reagents

- ✓ 5 mM GSH
- ✓ 25 mM H_2O_2
- ✓ 25 mM NaN_3
- ✓ Phosphate Buffer (0.05 mM, pH 7)
- ✓ 1.65 % HPO_{32}
- ✓ 0.4 M Na_2HPO_4
- ✓ 1 mM DTNB

Procedure

Tissue homogenate (approximately 0.5 mg protein) was incubated with 0.1 ml of 5mM GSH, 0.1 ml of 1. 25 mM H2O2, 0.1ml of 25 mM NaN3 and phosphate buffer (0.05 mM, pH 7) in a total volume of 2.5 ml at 37oC for 10 min. The reaction was stopped by adding 2 ml of 1.65 % HPO32- and the reaction mixture was centrifuged at 1500 rpm for 10 min. 2 ml of the supernatant was mixed with 2 ml 0.4 M Na2HPO4 and 1ml of 1mM DTNB. The absorbance of the yellow colored complex was measured at 412 nm after incubation for 10 min at 37oC against distilled water. A sample without the tissue homogenate processed in the same way was kept as non enzymatic reaction.

6.9 ANALYSIS OF NON ENZYMATIC ANTIOXIDANT PARAMETERS

6.9.1 Estimation of Reduced Glutathione (GSH) [240]

The most widely used method for the determination of GSH in biological samples is by Ellman reagent (DTNB), which reacts with sulfydryl compounds to give a relatively stable yellow color. This compound is water soluble and the color formed is proportional to the amount of GSH. To 0.5 ml of citrated blood, 0.5 ml of 5% trichloro acetic acid (TCA) solution was added to precipitate the proteins and centrifuged at 3000 rpm for 20 min. To 0.1 ml of supernatant, 1 ml of sodium phosphate buffer and 0.5 ml of DTNB reagent was added. The absorbance of the yellow color developed was measured at 412 nm (Ellman,1959).

Reagents

- ✓ TCA (5%)
- ✓ Phosphate buffer (0.2M, pH 8.0)
- ✓ DTNB (0.6mM in 0.2M phosphate buffer)
- ✓ Standard GSH (10nmoles/ml of 5% TCA)

Procedure

The supernatant (0.1ml) was made up to 1.0ml with 0.2M sodium phosphate buffer (pH 8.0). Standard GSH corresponding to concentrations ranging between 2 and 10 n moles were also prepared. Two ml of freshly prepared DTNB solution was added and the intensity of the yellow colour developed was measured in a spectrophotometer (Genesys 10-S, USA) at 412nm after 10 minutes. The values are expressed as n moles GSH/g sample.

6.8 HISTOPATHOLOGICAL STUDY [232]

Hematoxylin, a basic dye is oxidized to hematein with a mordant, a metallic ion such as the salts of aluminium. The positively charged aluminium-hematein complex combines with the negatively charged phosphate groups of the nucleic acids (DNA and RNA) forming blue/purple colour, which is characteristic of hematoxylin stains. Eosin is an acidic dye, which is considered to have a selective affinity for the basic parts of the cell, i.e., the cytoplasm. Thus, the hematoxylin and eosin (H & E) stain is used to demonstrate different structures of the tissue.

The various steps involved in the preparation of tissues for histological studies are as follows:

1. **Fixation**

In order to avoid tissue by the lysosomal enzymes and to preserve its physical and chemical structure, a bit of tissue from each organ was cut and fixed in bouin's fluid immediately after removal from the animal body. The tissues were fixed in bouin's fluid for about 24 hours. The tissues were then taken and washed in glass distilled water for a day to remove excess of picric acid.

2. **Dehydration**

The tissues were kept in the following solutions for an hour each; 30%, 50%, 70% and 100% alcohol. Inadequately dehydrated tissues cannot be satisfactorily infiltered with paraffin. At the same time over dehydration results in making the tissues brittle, which would be difficult for sectioning. So, careful precautions shoule be followed while performing the dehydration process.

3. Clearing

Dealcoholization or replacement of alcohol from the tissues with a clearing agent is called as clearing. Xylene was used as the clearing agent for one or two hours, two or three times. Since, the clearing agent is miscible with both dehydration and embedding agents, it permits paraffin to infilterate the tissues. So, the clearing was carried out as the next step after dehydration to permit tissue spaces to be filled with paraffin. The tissues were kept in the clearing agent till they become transparent and impregnated with xylene.

4. Impregnation

In this process the clearing agent xylene was placed by paraffin wax. The tissues were taken out of xylene and were kept in molten paraffin embedding bath, which consists of metal pots filled with molten wax maintained at about 50^{o}C. The tissues were given three changes in the molten wax at half an hour intervals.

5. Embedding

The paraffin wax used for embedding should be fresh and heated upto the optimum melting point at about 56–58^{o}C. A clear glass plate was smeared with glycerine. L-shaped mould was placed on it to from a rectangular cavity. The molten paraffin wax was poured and air bubbles were removed by using a hot needle. The tissue was placed in the paraffin and oriented with the surface to be sectioned. Then the tissue was pressed gently towards the glass plate to make settle uniformly with a metal pressing rod and allowed the wax to settle and solidity room temperature. The paraffin block was kept in cold water for cooling.

6. Section Cutting

Section cutting was done with a rotatory microtome. The excess of paraffin around the tissue was removed by trimming, leaving ½ cm around the tissue. Then the block was attached to the gently heated holder. Additional support was given by some extra wax, which was applied along the sides of the block. Before sectioning, all set screws holding the object holder and knife were hand tightened to avoid vibration. To produce uniform sections, the microtome knife was adjusted to the proper angle in the knife holder with only the cutting edge coming in contact with the paraffin block. The tissue was cut in the thickness range of about 7µm.

7. Flattening and Mounting of Sections

The procedure was carried out in tissue flotation warm water bath. The sections were spread on a warm water bath after they were detached from the knife with the help of hair brush. Dust free clean slides were coated with egg albumin over the whole surface. Required sections were spread on clean slide and kept at room temperature.

8. Staining of Tissue Sections

The sections were stained as follows; deparaffinization with xylene two times each for five minutes

Dehydration through descending grades of ethyl alcohol

- ✓ 100% alcohol (absolute) - 2 minute
- ✓ 90% alcohol - 1minute
- ✓ 50% alcohol - 1 minute

Staining with Ehrlich's Haemaoxylin was done for 15-20 minutes. Then the sectioned tissues were thoroughly washed in tap water for 10 minutes. Rinsed with distilled water and stained with Eosin. Dehydration again done with ascending grades of alcohol,

- ✓ 70% alcohol - 2 minute
- ✓ 90% alcohol - 2 minute
- ✓ 100%alcohol - 1 minute

Finally the tissues were cleared with xylene two times, each for about 3 minutes interval.

9. Mounting

On the stained slide, DPX mountant was applied uniformly and microglass cover slides were spread. The slides were observed in Nikon microscope and microphotographs were taken.

6.9 STATISTICAL ANALYSIS

Results were expressed as Mean ± SEM. The data were analyzed by using one way analysis of variance (ANOVA) followed by Tukey's Multiple Comparison Test. P value < 0.05 was considered as statistically significant. Data were processed with graph pad prism 5.0 software.

7. RESULTS

The *Tamrindus indica* Linn (Family: Fabaceae) was selected, on the basis of ethanobotanical information which reveals its uses against one of the most hazardous diseases.

Tamrindus indica Linn is grown almost in the Tamilnadu and Kerala. This medicinal plant possesses many pharmacological activities based upon the phytochemical constituents of it.

The fruit pulp of *Tamrindus indica* Linn was used to treat Nephrotoxic condition. Detailed pharmacognostical and preliminary phytochemical investigations were taken to prove its appropriate identification and rationalized its use as a drug of therapeutic importance.

Extracting values of crude drug were useful for their evaluation. Further, these values indicated the nature of the constituents present in the crude drug. The small pieces of the fruit pulps of *Tamrindus indica* Linn was subjected for continuous hot percolation using 95% ethanol as a solvent using a Soxhlet apparatus. The percentage of extract was calculated. The result was tabulated in the table-5.

Table-5

Results of the Percentage yield of *EETI*

Drug	*Tamarindus indica* Linn.
Solvent	Ethanol
Colour	Dark Brownish
Consistency	Semi solid
Percentage yield	22 % w/w

7.1 Preliminary phytochemical studies

Table no-5: Results of the Preliminary Phytochemical Constituents present in ethanolic extract of *Tamarindus indica* Linn.

Table-6

Results of preliminary phytochemical analysis of *EETI*

Phyto-constituents	***Tamarindus indica* Linn**
Carbohydrate	Present
Reducing sugar	Present
Tannins	Present
Flavonoids	Present
Anthroquinone	Present
Saponins	Present
Alkaloids	Present
Glycosides	Present
Terpenes	Present
Phytosterols	Absent

RESULT

Ethanolic extract of the whole plants of *Tamarindus indica* Linn (*EETI*) was subjected to various phytochemical tests, which showed the presence of carbohydrates, reducing sugars, glycosides, tannins, flavonoids, Anthroquinone, Saponins, Alkaloids, Glycosides and Terpenes.

7.2 Assessment of general biochemical parameters

7.2.1 Assessment of urine volume

The effects of the different doses of ethanolic extract of Tamarindus indica Linn on urine volume.

Table-7

Results of the effect of *EETI* on urine volume in cisplatin induced Nephrotoxic rats

Groups	Drug Treatment	Urine Volume
I	Normal Control (0.5% DMSO)	10.85± 0.223
II	Nephrotoxic Control Cisplatin (0.75%)	5.90± 0.762
III	Reference Control Cisplatin (0.75%) + Lipoic acid (50mg/kg)	10.81± 0.24***
IV	Cisplatin (0.75%) + *EETI* (200mg/kg)	9.17± 0.40*
V	Cisplatin (0.75%) + *EETI* (400mg/kg)	9.70± 0.28***

Values were given in Mean ±SEM;

*P<0.05, ** P<0.01 and** *P<0.001 Vs Nephrotoxic Control

Diagrammatic representation of *EETI* on urine volume in cisplatin induced Nephrotoxic rats

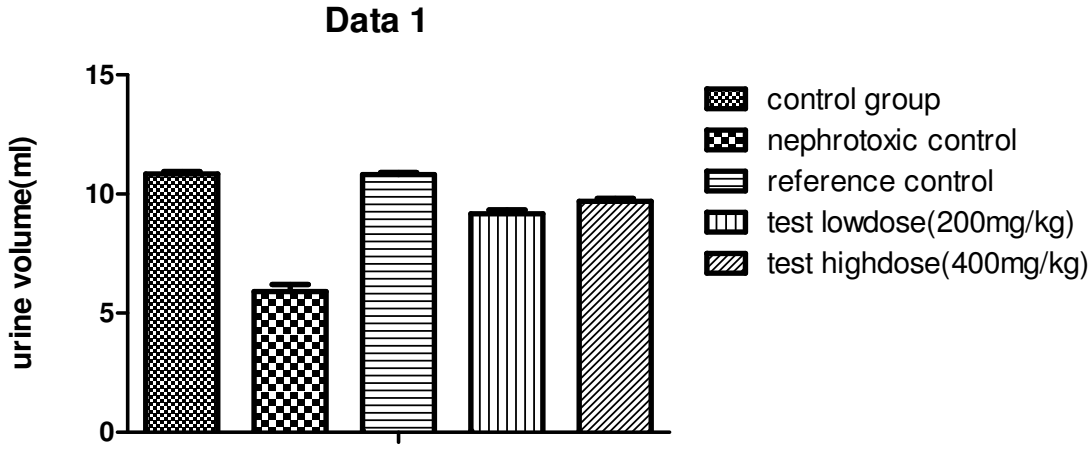


Fig. No: 8

RESULTS

The urine volume were measured were showed in Table no-7 and Fig. no-8.

The Nephrotoxic control (Group 2) showed significant decrease in urine volume when compared to the normal control (Group1).

Standard (Group 3) showed statistically significant increase in urine volume when compared to Nephrotoxic control (Group 2).

EETI 200 mg/kg treated (Group 4) showed statistically significant increase in urine volume when compared to the Nephrotoxic control (Group 2).

EETI 400mg/kg treated (Group 5) showed statistically significant increase in urine volume when compared to the Nephrotoxic control (Group 2).

7.2.2 Assessment of Body weight

The effects of the different doses of ethanolic extract of Tamarindus indica Linn on body weight.

Table-8

Results of the effect of *EETI* on Body weight in cisplatin induced Nephrotoxic rats

Groups	Drug Treatment	Body weight
I	Normal Control (0.5% DMSO)	250± 3.406
II	Nephrotoxic Control Cisplatin (0.75%)	159.33± 2.658
III	Reference Control Cisplatin (0.75%) + Lipoic acid (50mg/kg)	234.83± 4.355***
IV	Cisplatin (0.75%) + *EETI* (200mg/kg)	205.83± 3.43*
V	Cisplatin (0.75%) + *EETI* (400mg/kg)	222± 3.742***

Values were given in Mean ±SEM;

*P<0.05, ** P<0.01 and** *P<0.001 Vs Nephrotoxic Control

Diagrammatic representation of *EETI* on serum creatinine level on cisplatin induced Nephrotoxicity in rats

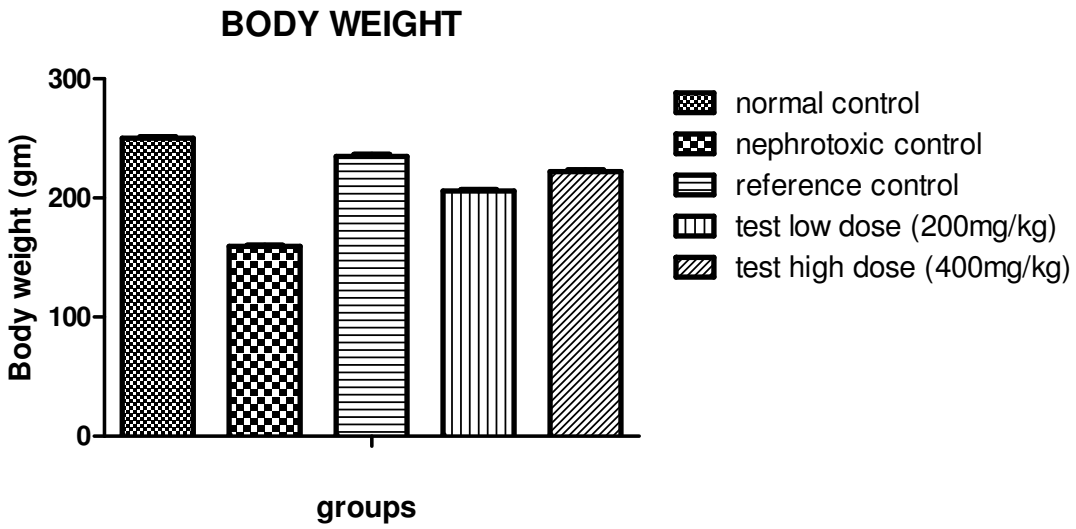


Fig. No: 9

RESULTS

The body weight were measured were showed in Table no-8 and Fig. no-9.

The Nephrotoxic control (Group 2) showed significant decrease in body weight when compared to the normal control (Group1).

Standard (Group 3) showed statistically significant increase in body weight when compared to Nephrotoxic control (Group 2).

EETI 200 mg/kg treated (Group 4) showed statistically significant increase in body weight when compared to the Nephrotoxic control (Group 2).

EETI 400mg/kg treated (Group 5) showed statistically significant increase in body weight when compared to the Nephrotoxic control (Group 2).

7.3 Assessment of serum biochemical parameters

7.3.1 Serum creatinine level

The effects of the different doses of ethanolic extract of *Tamarindus indica* Linn on serum creatinine level.

TABLE-9

Results of the effect of the *EETI* on serum Creatinine on Cisplatin induced Nephrotoxicity in rats

Groups	Drug Treatment	Serum creatinine
I	Normal Control (0.5% DMSO)	0.67± 0.055
II	Nephrotoxic Control Cisplatin (0.75%)	4.77± 0.131
III	Reference Control Cisplatin (0.75%) + Lipoic acid (50mg/kg)	0.75± 0.020***
IV	Cisplatin (0.75%) + *EETI* (200mg/kg)	1.47± 0.206*
V	Cisplatin (0.75%) + *EETI* (400mg/kg)	0.82± 0.062***

Values were given in Mean ±SEM;

*P<0.05, ** P<0.01 and** *P<0.001 Vs Nephrotoxic Control

Diagrammatic representation of *EETI* on serum creatinine level on cisplatin induced Nephrotoxicity in rats

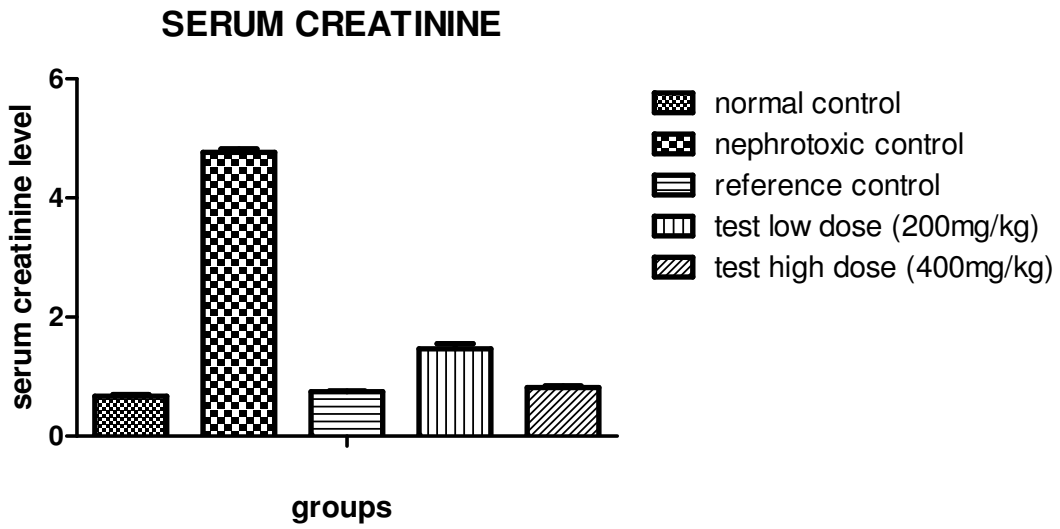


Fig. no-10

RESULTS

The serum creatinine were measured were showed in Table no-9 and Fig. no-10.

The Nephrotoxic control (Group 2) showed significant increase in serum creatinine level when compared to the normal control (Group1).

Standard (Group 3) showed statistically significant decrease in serum creatinine level when compared to Nephrotoxic control (Group 2).

EETI 200 mg/kg treated (Group 4) showed statistically significant decrease in serum creatinine level when compared to the Nephrotoxic control (Group 2).

EETI 400mg/kg treated (Group 5) showed statistically significant decrease in serum creatinine level when compared to the Nephrotoxic control (Group 2).

7.3.2 Serum Blood urea nitrogen (BUN)

The effects of the different doses of ethanolic extract of *Tamarindus indica* Linn on serum Blood urea nitrogen (BUN) level.

Table-10

Results of the effect of the *EETI* on serum Blood urea nitrogen on Cisplatin induced Nephrotoxicity in rats

Groups	Drug Treatment	Serum blood urea nitrogen (BUN)
I	Normal Control (0.5% DMSO)	23.66± 0.505
II	Nephrotoxic Control Cisplatin (0.75%)	58.77± 0.792
III	Reference Control Cisplatin (0.75%) + Lipoic acid (50mg/kg)	24.15± 0.50***
IV	Cisplatin (0.75%) + *EETI* (200mg/kg)	30.02± 0.94*
V	Cisplatin (0.75%) + *EETI* (400mg/kg)	24.55± 0.55***

Values were given in Mean ±SEM;

*P<0.05, ** P<0.01 and** *P<0.001 Vs Nephrotoxic Control

Diagrammatic representation of *EETI* on serum Blood urea nitrogen parameters on cisplatin induced Nephrotoxicity in rats

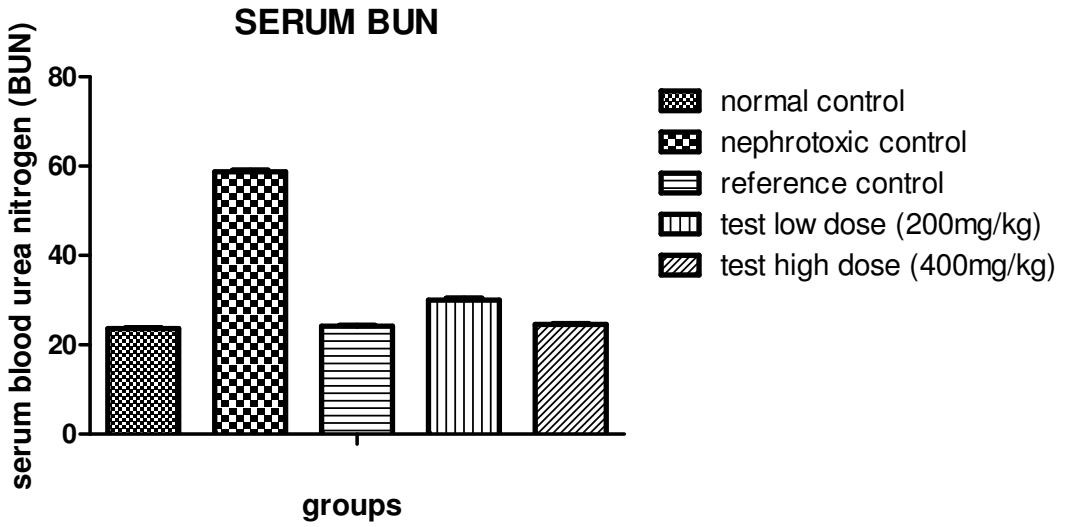


Fig. No: 11

RESULTS

The serum Blood urea nitrogen (BUN) level were measured were showed in Table no-10 and Fig. no-11.

The Nephrotoxic control (Group 2) showed significant increase in serum Blood urea nitrogen (BUN) level when compared to the normal control (Group1).

Standard (Group 3) showed statistically significant decrease in serum Blood urea nitrogen (BUN) level when compared to Nephrotoxic control (Group 2).

EETI 200 mg/kg treated (Group 4) showed statistically significant decrease in serum Blood urea nitrogen (BUN) level when compared to the Nephrotoxic control (Group 2).

EETI 400mg/kg treated (Group 5) showed statistically significant decrease in serum Blood urea nitrogen (BUN) level when compared to the Nephrotoxic control (Group 2).

7.4 Assessment of urine biochemical parameters

7.4.1 Assessment of creatinine clearence

The effects of the different doses of ethanolic extract of *Tamarindus indica* Linn on creatinine clearance.

Table-11

Results of the effect of *EETI* on creatinine clearence in cisplatin induced Nephrotoxic rats

Groups	Drug Treatment	Creatinine clearance
I	Normal Control (0.5% DMSO)	19.80± 1.302
II	Nephrotoxic Control Cisplatin (0.75%)	5.05± 0.445
III	Reference Control Cisplatin (0.75%) + Lipoic acid (50mg/kg)	18.265± 0.512***
IV	Cisplatin (0.75%) + *EETI* (200mg/kg)	14.74± 0.746*
V	Cisplatin (0.75%) + *EETI* (400mg/kg)	17.20± 1.146***

Values were given in Mean ±SEM;

*P<0.05, ** P<0.01 and** *P<0.001 Vs Nephrotoxic Control

Diagrammatic representation of *EETI* on creatinine clearance in cisplatin induced Nephrotoxic rats

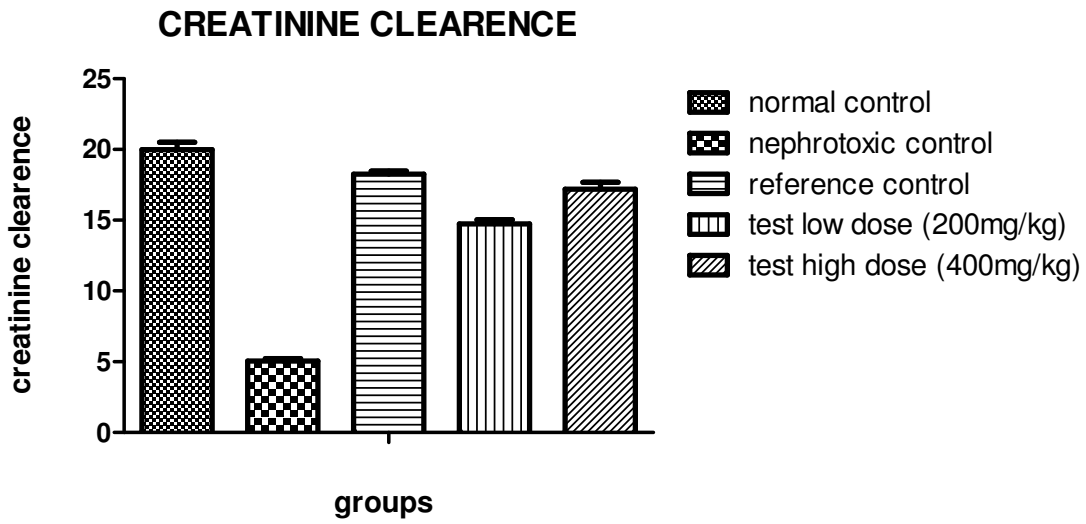


Fig. no- 12

RESULTS

The creatinine clearence were measured were showed in Table no-11 and Fig. no-12.

The Nephrotoxic control (Group 2) showed significant decrease in creatinine clearence when compared to the normal control (Group1).

Standard (Group 3) showed statistically significant increase in creatinine clearence when compared to Nephrotoxic control (Group 2).

EETI 200 mg/kg treated (Group 4) showed statistically significant increase in creatinine clearence when compared to the Nephrotoxic control (Group 2).

EETI 400mg/kg treated (Group 5) showed statistically significant increase in creatinine clearence when compared to the Nephrotoxic control (Group 2).

7.5 Assessment of oxidative stress parameter

7.5.1 Assessment of Malondialdehyde (MDA)

The effects of the different doses of ethanolic extract of *Tamarindus indica* Linn on malondialdehyde (MDA).

Table-12

Results of the effect of *EETI* on Malondialdehyde (MDA) in cisplatin induced Nephrotoxic rats

Groups	Drug Treatment	Malondialdehyde (MDA)
I	Normal Control (0.5% DMSO)	7.61± 0.470
II	Nephrotoxic Control Cisplatin (0.75%)	15.44± 0.409
III	Reference Control Cisplatin (0.75%) + Lipoic acid (mg/kg)	7.86± 0.118***
IV	Cisplatin (0.75%) + *EETI* (200mg/kg)	8.77± 0.427**
V	Cisplatin (0.75%) + *EETI* (400mg/kg)	7.66± 0.238***

Values were given in Mean ±SEM;

*P<0.05, ** P<0.01 and** *P<0.001 Vs Nephrotoxic Control

Diagrammatic representation of *EETI* on Malondialdehyde (MDA) in cisplatin induced Nephrotoxic rats

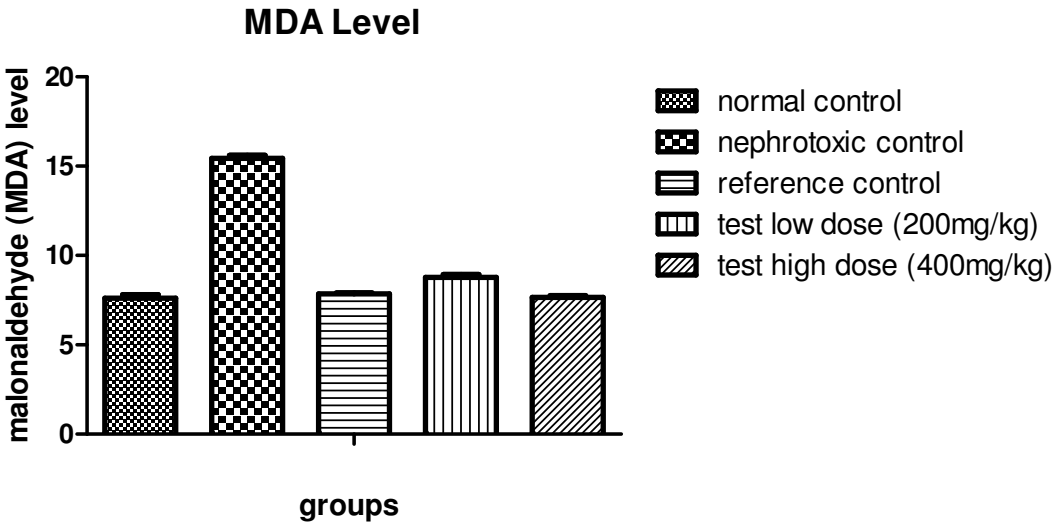


Fig. no- 13

RESULTS

The malondialdehyde (MDA) were measured were showed in Table no-12 and Fig. no-13.

The Nephrotoxic control (Group 2) showed significant increase in malondialdehyde (MDA) when compared to the normal control (Group1).

Standard (Group 3) showed statistically significant decrease in malondialdehyde (MDA) when compared to Nephrotoxic control (Group 2).

EETI 200 mg/kg treated (Group 4) showed statistically significant decrease in malondialdehyde (MDA) when compared to the Nephrotoxic control (Group 2).

EETI 400mg/kg treated (Group 5) showed statistically significant decrease in malondialdehyde (MDA) when compared to the Nephrotoxic control (Group 2).

7.6 Assessment of enzymatic antioxidant parameters

7.6.1 Assessment of superoxide dismutase (SOD)

The effects of the different doses of ethanolic extract of *Tamarindus indica* Linn on superoxide dismutase (SOD).

Table-13

Results of the effect of *EETI* on superoxide dismutase (SOD) in cisplatin induced Nephrotoxic rats

Groups	Drug Treatment	Superoxide dismutase (SOD)
I	Normal Control (0.5% DMSO)	19.56± 0.591
II	Nephrotoxic Control Cisplatin (0.75%)	7.53± 0.423
III	Reference Control Cisplatin (0.75%) + Lipoic acid (50mg/kg)	18.50± 0.44***
IV	Cisplatin (0.75%) + *EETI* (200mg/kg)	11.89± 0.303*
V	Cisplatin (0.75%) + *EETI* (400mg/kg)	15.57± 0.375***

Values were given in Mean ±SEM;

*P<0.05, ** P<0.01 and** *P<0.001 Vs Nephrotoxic Control

Diagrammatic representation of *EETI* on superoxide dismutase (SOD) in cisplatin induced Nephrotoxic rats

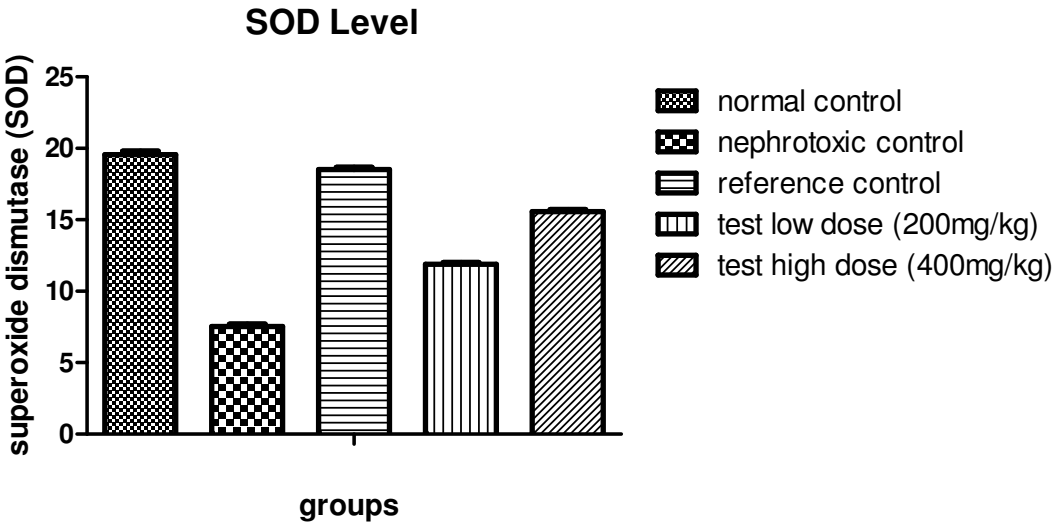


Fig. no- 14

RESULTS

The superoxide dismutase (SOD) were measured were showed in Table no-13 and Fig. no-14.

The Nephrotoxic control (Group 2) showed significant decrease in superoxide dismutase (SOD) when compared to the normal control (Group1).

Standard (Group 3) showed statistically significant increase in superoxide dismutase (SOD) when compared to Nephrotoxic control (Group 2).

EETI 200 mg/kg treated (Group 4) showed statistically significant increase in superoxide dismutase (SOD) when compared to the Nephrotoxic control (Group 2).

EETI 400mg/kg treated (Group 5) showed statistically significant increase in superoxide dismutase (SOD) when compared to the Nephrotoxic control (Group 2).

7.6.2 Assessment of Catalase (CAT)

The effects of the different doses of ethanolic extract of *Tamarindus indica* Linn on Catalase (CAT).

Table-14

Results of the effect of *EETI* on Catalase (CAT) in cisplatin induced Nephrotoxic rats

Groups	Drug Treatment	Catalase (CAT)
I	Normal Control (0.5% DMSO)	220.31± 0.52
II	Nephrotoxic Control Cisplatin (0.75%)	104.94± 0.37
III	Reference Control Cisplatin (0.75%) + Lipoic acid (50mg/kg)	200.03± 0.612***
IV	Cisplatin (0.75%) + *EETI* (200mg/kg)	158.39± 4.091**
V	Cisplatin (0.75%) + *EETI* (400mg/kg)	181± 0.265***

Values were given in Mean ±SEM;

*P<0.05, ** P<0.01 and** *P<0.001 Vs Nephrotoxic Control

Diagrammatic representation of *EETI* on Catalase (CAT) in cisplatin induced Nephrotoxic rats

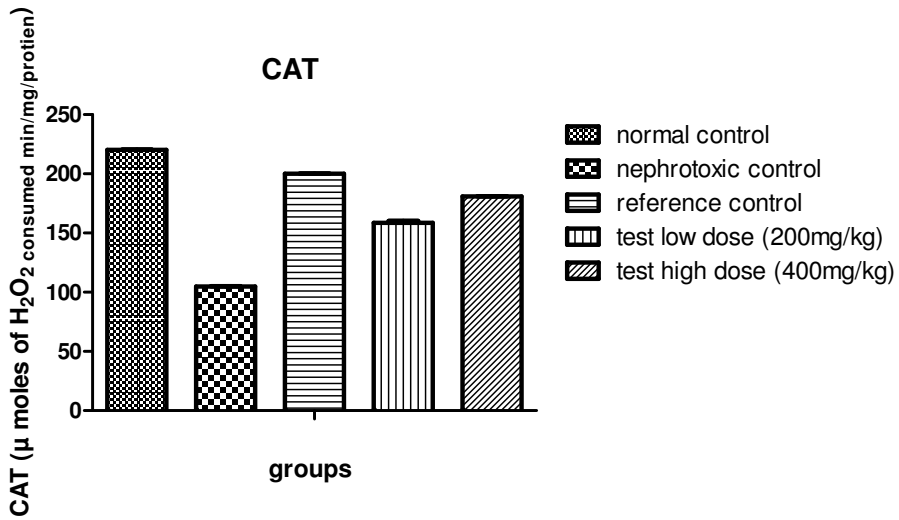


Fig. no- 15

RESULTS

The Catalase (CAT) were measured were showed in Table no-14 and Fig. no-15.

The Nephrotoxic control (Group 2) showed significant decrease in Catalase (CAT) when compared to the normal control (Group1).

Standard (Group 3) showed statistically significant increase in Catalase (CAT) when compared to Nephrotoxic control (Group 2).

EETI 200 mg/kg treated (Group 4) showed statistically significant increase in Catalase (CAT) when compared to the Nephrotoxic control (Group 2).

EETI 400mg/kg treated (Group 5) showed statistically significant increase in Catalase (CAT) when compared to the Nephrotoxic control (Group 2).

7.6.3 Assessment of Glutathione peroxidise (GPx)

The effects of the different doses of ethanolic extract of *Tamarindus indica* Linn on Glutathione peroxidise (GPx).

Table-15

Results of the effect of *EETI* on Glutathione peroxidise (GPx) in cisplatin induced Nephrotoxic rats

Groups	Drug Treatment	Glutathione peroxidise (GPx)
I	Normal Control (0.5% DMSO)	23.29± 0.45
II	Nephrotoxic Control Cisplatin (0.75%)	14.48± 0.448
III	Reference Control Cisplatin (0.75%) + Lipoic acid (50mg/kg)	21.39± 0.37***
IV	Cisplatin (0.75%) + *EETI* (200mg/kg)	16.33± 0.399**
V	Cisplatin (0.75%) + *EETI* (400mg/kg)	19.26± 0.228***

Values were given in Mean ±SEM;

*P<0.05, ** P<0.01 and** *P<0.001 Vs Nephrotoxic Control

Diagrammatic representation of *EETI* on Glutathione peroxidise (GPx) in cisplatin induced Nephrotoxic rats

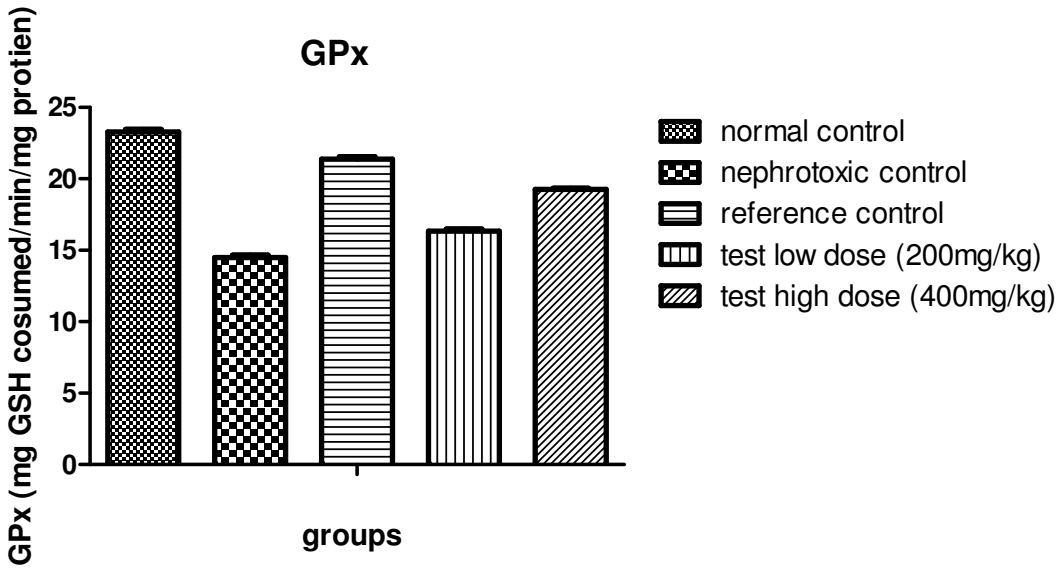


Fig. no- 16

RESULTS

The Glutathione peroxidise (GPx) were measured were showed in Table no-15 and Fig. no-16.

The Nephrotoxic control (Group 2) showed significant decrease in Glutathione peroxidise (GPx) when compared to the normal control (Group1).

Standard (Group 3) showed statistically significant increase in Glutathione peroxidise (GPx) when compared to Nephrotoxic control (Group 2).

EETI 200 mg/kg treated (Group 4) showed statistically significant increase in Glutathione peroxidise (GPx) when compared to the Nephrotoxic control (Group 2).

EETI 400mg/kg treated (Group 5) showed statistically significant increase in Glutathione peroxidise (GPx) when compared to the Nephrotoxic control (Group 2).

7.7 Assessment of non-enzymatic antioxidant parameter

7.7.1 Assessment of Reduced glutathione (GSH)

The effects of the different doses of ethanolic extract of *Tamarindus indica* Linn on Reduced glutathione (GSH).

Table-16

Results of the effect of *EETI* on Reduced glutathione (GSH) in cisplatin induced Nephrotoxic rats

Groups	Drug Treatment	Reduced glutathione (GSH)
I	Normal Control (0.5% DMSO)	20.15± 0.776
II	Nephrotoxic Control Cisplatin (0.75%)	8.28± 0.201
III	Reference Control Cisplatin (0.75%) + Lipoic acid (50mg/kg)	18.47± 0.488***
IV	Cisplatin (0.75%) + *EETI* (200mg/kg)	14.37± 0.280**
V	Cisplatin (0.75%) + *EETI* (400mg/kg)	16.33± 0.566***

Values were given in Mean ±SEM;

*P<0.05, ** P<0.01 and** *P<0.001 Vs Nephrotoxic Control

Diagrammatic representation of *EETI* on Reduced glutathione (GSH) in cisplatin induced Nephrotoxic rats

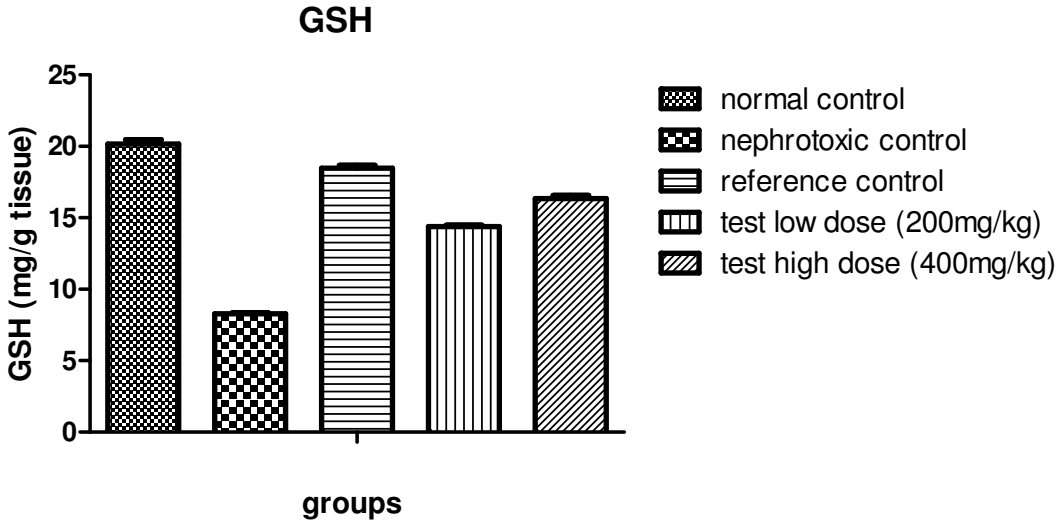


Fig. no- 17

RESULTS

The Reduced glutathione (GSH) were measured were showed in Table no-16 and Fig. no-17.

The Nephrotoxic control (Group 2) showed significant decrease in Reduced glutathione (GSH) when compared to the normal control (Group1).

Standard (Group 3) showed statistically significant increase in Reduced glutathione (GSH) when compared to Nephrotoxic control (Group 2).

EETI 200 mg/kg treated (Group 4) showed statistically significant increase in Reduced glutathione (GSH) when compared to the Nephrotoxic control (Group 2).

EETI 400mg/kg treated (Group 5) showed statistically significant increase in Reduced glutathione (GSH) when compared to the Nephrotoxic control (Group 2).

7.8 HISTOPATHOLOGICAL STUDIES

a) Normal group

Section of the kidney of normal control rat showed,

- Arrangement of nephrotic bundles appears normal, both cortex and medulla appears normal.
- Normal glomerular structure with regularly arranged podocytes was observed.
- No signs of degeneration and edema and no signs of inflammation like glomerulonephritis.
- Proximal and Distal convoluted tubule appears normal and intact.
- No signs of karyolysis.

b) Nephrotoxic group

Section of the kidney of Nephroprotective control rat showed the following,

- Appearance of coagulative and diffused necrosis
- Severe Glomerulonephritis- Glomerular condensation and appearance of inflammatory cells
- Marked signs of hemorrhage, edema and narrowed renal arterioles.

c) Standard group

Section of the kidney of lipoic acid treated group rat showed normal histology of kidney and a20bsence of necrosis.

d) Extract treated groups

Section of the kidney treated with low dose (200mg/kg) of *EETI* showed

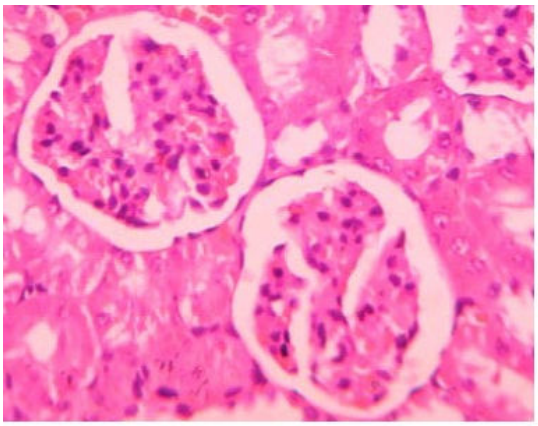
- Moderate tubular degeneration with mild edema and
- Necrotic changes with swollen tubular epithelium.

Section of the kidney treated with high dose (400mg/kg) of *EETI* showed

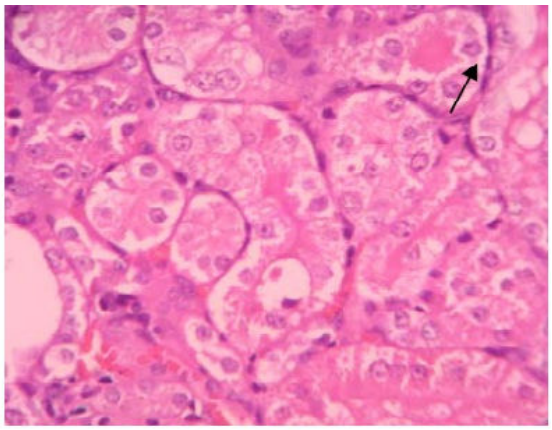
- Moderated signs of regeneration with occurrence of chromatolysis was observed in the tubular structure and
- Stripping of tubular epithelium with inter tubular edema.

HISTOPATHOLOGICAL STUDIES

Group I

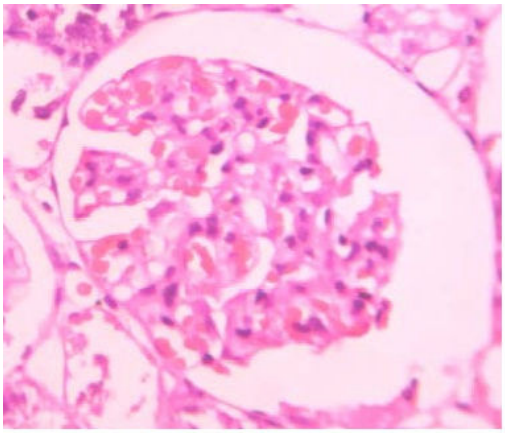


Control- 1

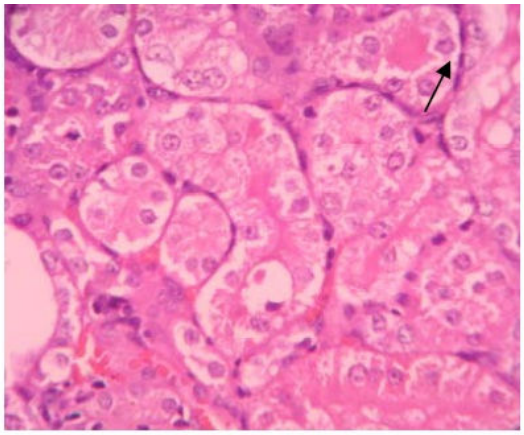


Control -2

Group II

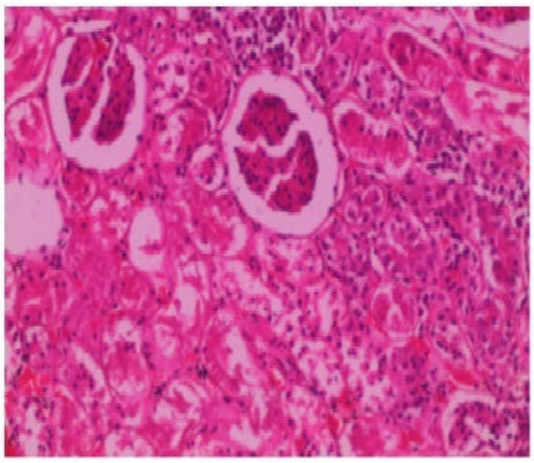


Cisplatin induced 1

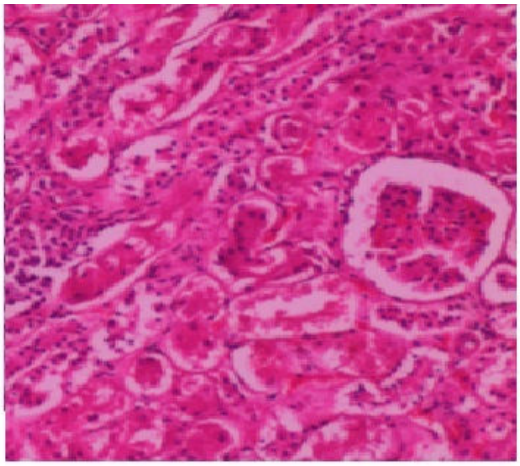


Cisplatin induced 2

Group III

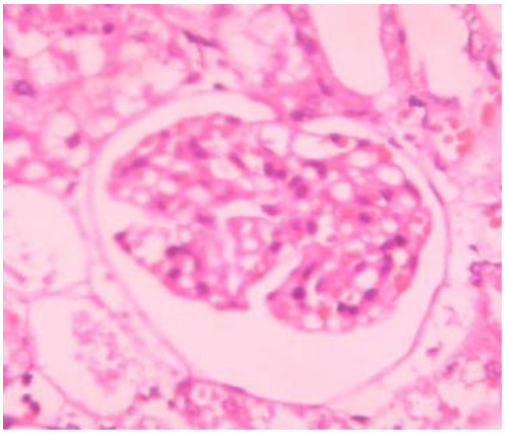


Standard (lipoic acid) + Cisplatin 1

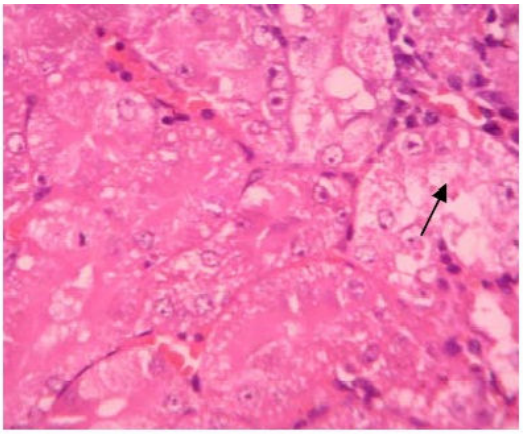


Standard (lipoic acid) + Cisplatin 2

Group IV

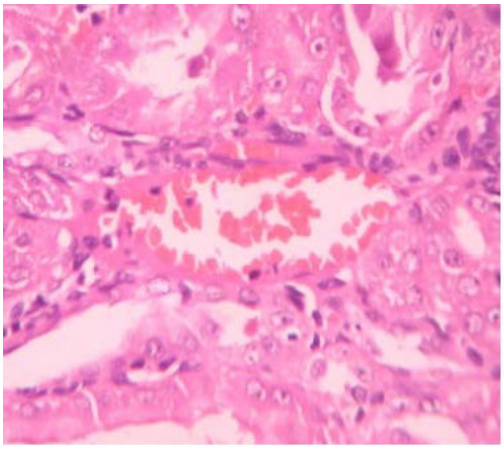


Plant extract 200mg + Cisplatin - 1(Kidney)

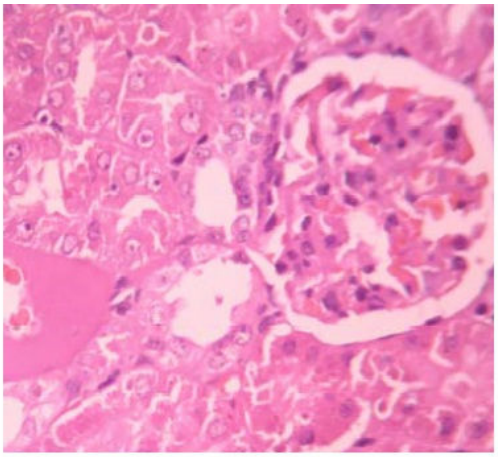


Plant extract 200mg + Cisplatin - 2 (Kidney)

Group V



Plant extract (400mg) + Cisplatin (Kidney) 1



Plant extract (400mg) + Cisplatin (Kidney) - 2

Fig. no- 18: Photomicrographs of kidney tissue section Group I- Normal control, Group II- Nephrotoxic Control, Group III- Reference Control, Group IV- *EETI* (200mg/kg), Group V- *EETI* (400mg/kg)

8. DISCUSSION

Nephrotoxicity is a common clinical syndrome defined as a rapid decline in renal function resulting in abnormal retention of serum creatinine and blood urea, which must be excreted.

There are few chemical agents to treat acute renal failure. Studies reveal back synthetic nephroprotective agents have adverse effect besides reduce nephrotoxicity.

There is a growing interest of public in traditional medicine, particularly in the treatment of nephrotoxicity partly because of limited choice in the pharmacotherapy. Many plants have been used for the treatment of kidney failure in traditional system of medicine throughout the world. Indeed along with the dietary measures, plant preparation formed the basis of treatment of disease until the introduction of allopathic medicine.

Ethnomedicinal plants can be used to help forestall the need of dialysis by treating the causes and effect of renal failure, as well as reducing the many adverse effect of dialysis.

The phytochemicals found to be present in the fruit pulp extract are the flavanoids, terpenoids, alkaloids, tannins, saponins and anthraquinones. Among them tannins, triterpenoids, flavanoids and saponins could be responsible for antioxidant property as these phytoconstituents are already reported to have antioxidant activity.[216]

Acute toxicity studies revealed the non-toxic nature of the ethanolic extract of *Tamarindus indica* Linn. There was no lethality or any toxic reactions found with high dose (2000 mg/kg body weight) till the end of the study. According to the OECD 423 guidelines (Acute Oral Toxicity: Acute Toxic Classic Method), an LD_{50} dose of 2000 mg/kg and above was considered as unclassified so the ethanolic extract of *Tamarindus indica* Linn was found to be safe.

Cisplatin causes damage to nuclear and mitochondrial DNA and production of reactive oxygen species (ROS) which lead to activation of both mitochondrial and

non mitochondrial pathways of apoptosis and necrosis. Mitochondrial energetic are also disrupted by cisplatin and may contribute to nephrotoxicity.[47]

In present study, the rats treated with single dose of Cisplatin shown marked reduction of body weight as compared to normal group also caused a marked reduction of glomourular filtration rate, which is accompanied by increase in serum creatinine level and declain in creatinine clearence indicating induction of acute renal failure.[241] with *Tamarindus indica* Linn at the dose level of 200 and 400 mg/kg body weight for 15 days significantly lowered the serum level of creatinine with a significant weight gain, increased urine output and creatinine clearence when compared with the nephrotoxic control group.

Cisplatin administration to control rats produced a typical pattern of nephrotoxicity which was manifested by marked increase in serum blood urea nitrogen (BUN).[65] *Tamarindus indica* Linn supplementation to Cisplatin treated rats recorded decrement in levels of blood urea nitrogen (BUN) in plasma.

The elevated level of malondialdehyde (MDA), a marker of lipid preroxidation, indicates increased free radical generation in the Cisplatin induced nephrotoxicity. Cisplatin induced increment in malondialdehyde (MDA) content of plasma was significantly prevented by *Tamarindus indica* Linn treatment in the present study. Therefore, the significantly lower levels of malondialdehyde (MDA) in the kidney tissues of treated groups as compared with the Cisplatin group indicate attenuation of lipid peroxidation. This was probably due to less damage by oxygen free radicals with *Tamarindus indica* Linn. The involvement of oxygen free radicals in tissue injury is well established.[66]

Decrement in activity levels of renal Superoxide dismutase (SOD), Catalase (CAT) and Reduced Glutathione (GSH) following Cisplatin treatment are in accordance with previous report on Cisplatin induced suppression of endogenous enzymatic antioxidant machinery.[66] *Tamarindus indica* Linn treatment efficiently prevented Cisplatin induced decrease in activity levels of superoxide dismutase (SOD), Catalase (CAT) and Reduced Glutathione (GSH).[242] A relationship between nephrotoxicity and oxidative stress has been confirmed in many experimental models.

Biological systems protect themselves against the damaging effects of activated species by several means. These include free radical scavengers and chain reaction terminators such as GPX system. Glutathione peroxidise (GPx) is a seleno-enzyme two third of which is present in the cytosol and one-third in the mitochondria, It catalyses the reaction of hydro-peroxides with reduced Glutathione to form Glutathione disulphide (GSSG) and the reduction product of the hydro-peroxide.[243]Effect of *Tamarindus indica* Linn on Glutathione peroxidise (GPx) in experimental rats study were significantly reduced in cisplatin treated rats than in the experimental control rats. Decrement in the activity of renal GPx following cisplatin treatment are due to suppression of endogenous enzymatic antioxidant machinery. Supplementation with *Tamarindus indica* Linn to Cisplatin treated rats resulted in near normal activity of glutathione peroxidise (GPx).

Based on the above results, it was concluded that *Tamarindus indica* Linn exerted statistically significant Nephroprotective activity against cisplatin induced Nephrotoxic rats.

9. SUMMARY AND CONCLUSION

The present study was undertaken to scientifically evaluate the nephroprotective activity of the ethanolic extract of fruit pulps of *Tamarindus indica* Linn.

The phytochemical investigation revealed the presence of carbohydrate, alkaloids, flavanoids, glycosides, saponins, tannins, phenols and anthroquinone in *EETI*.

The administration of cisplatin during experimentation is effectively induced apoptosis and necrosis, which was similar to acute renal failure in human. Therefore it is an effective and an ideal model for nephrotoxicity research.

The evaluation of renal parameters on nephrotoxic rats with *EETI* showed significantly elevate the attenuated body weight, urine volume, creatinine clearance and significant reduce in elevated serum creatinine level, which supports its Nephroprotective activity.

The cisplatin induced rats showed elevated levels of serum blood urea nitrogen (BUN) and lipid peroxidation parameter like malondialdehyde (MDA) which was significantly decreased with treatment of *EETI*, which proves it having Nephroprotective activity.

The Nephrotoxic rats also showed the reduced levels of enzymatic antioxidant like sulphoxide dismutase (SOD), glutathione peroxidise (GPx) and Catalase (CAT), and non-enzymatic antioxidant like Reduced glutathione (GSH), which was significantly increased with treatment of *EETI*, which showed its antioxidant activity due to the Flavonoids which is present in the extract.

Histopathological studies on isolated kidney revealed that the *EETI*, reversed the kidney damage and also restored normal kidney architecture.

In summary, the fruit pulp of *Tamarindus indica* Linn in an ethanolic extract showed statistically significant nephroprotective activity.

The plant extract proved to have nephroprotective potentials may because of its known flavonoid contents and antioxidant properties.

There is a scope for further investigation on the histopathology of liver and spleen and clinical studies that are required to elucidate the active phytoconstituents with potent nephroprotective activity.

10. FUTURE PROSPECTIVES

1. There is a scope for further investigation on the histopathology of liver and spleen, and clinical studies that are required to elucidate the active phytoconstituents with potent Nephroprotective activity.
2. A lead molecule having the Nephroprotective activity can be isolated from the ethanolic extract of the fruit pulps of *Tamarindus indica* Linn.
3. A Suitable formulation of the isolated lead molecule can be designed.
4. The formulated compound can be subjected to clinical trials in healthy human volunteers or diseased persons.
5. The formulated compound can be put for patency and after that it can be marketed.

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